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PHYTOPATHOLOGY

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MOSAIC AND LEAF CURL (YELLOWS) OF THE CULTIVATED RED RASPBERRY

W. H. Rankin and J. F. Hockey

After two years spent in a preliminary investigation (mostly observational by force of circumstances) of the disease which has been known as raspberry yellows, it is believed that a progress report is warranted. The previous literature on yellows is meager and confined chiefly to brief descriptions of the diseased plant, the suspected causes of the disease and suggested control measures. The possible causes suspicioned have included insects, fungi, bacteria, excessive soil water and unbalanced nutrition. Green (6) in 1895 states that for 5 years a disease known as leaf curl has seriously affected almost every variety of red raspberry on the grounds of the University Farm (Minnesota), and that its ravages were very wide spread in that district. Stewart (11) in 1902 described a serious disease of the variety Marlboro in New York State which he called yellows, because the reaction of the plant suggested peach yellows. Clinton (2) in 1903 briefly states that yellows as described by Stewart is present in Connecticut and is more severe on soils poor in nitrogen. Melchers (7) in 1914 described leaf curl which he believed to be the same as yellows. He gives many facts derived from observations in Ohio regarding its importance and possible cause. He also attempts to separate yellows or leaf curl as a disease distinct from other raspberry diseases which are characterized by a "yellowing" or sickly foliage. Clinton (3) in 1915 included yellows of raspberries in his discussion of mosaic diseases and briefly described the affected plants. Weiss[1] in 1918 described leaf curl from observations made in Minnesota and Manitoba. He also attempted various methods of inoculating healthy plants in the green house and in the field, and studied the effect of transplanting to different types of soil and the influence of different amounts of soil water. He did not succeed in transmitting

[1] Weiss, Freeman. A manuscript report on work done at the University of Minnesota, a copy of which was kindly furnished to the writers by Mr. Weiss.

the disease or in finding any connection between the type of soil and the amount of soil water in causing or relieving the symptoms of leaf curl. Dickson (5) in 1921 states that it is possible that mosaic and curl or yellows have been confused and that it remains to be seen whether or not curl is an advanced stage of raspberry mosaic. Several compiled bulletins and circulars describe yellows or leaf curl (used synonymously) giving the symptoms of both the mosaic and leaf curl as characteristic of the disease.

The above brief review of the literature on yellows or leaf curl is sufficient to call attention to two facts; first, that practically nothing is known except the symptoms and second, that there probably are two distinct diseases, one a mosaic and the other a leaf curl. As a result of the facts now available and reported below it may be stated that it is practically certain that Stewart described the mosaic and called it yellows, and that Melchers and Weiss were working with leaf curl alone, but confused the symptoms of leaf curl with the published descriptions of yellows and used the names as synonyms. The two diseases mosaic and leaf curl are distinct. Since the name "mosaic" has been very generally adopted for a type of disease with which Stewart's "yellows" conforms in all important respects and especially on account of the confusion which has occurred it might be better to abandon entirely the name "yellows."

Like other mosaic and leaf rolling or curling diseases these two raspberry diseases are difficult to describe fully in all their symptoms. The initial symptoms of the mosaic are more easily recognizable in some varieties than in others, and, under certain weather conditions the characteristic foliage mottling is more or less obscured. In these respects raspberry mosaic is subject to many of the uncertainties as regards diagnosis that are found in potato mosaic. On the other hand raspberry leaf curl, which has been found to be similar in many respects to potato leaf roll, has such well defined symptoms that one rarely need be uncertain of its diagnosis.

The work reported below was confined largely to the intensive fruit district of the Niagara Peninsula in Ontario. This district extends along the south shore of Lake Ontario from the Niagara River to the western end of the lake and for several miles along the northwestern shore. The red raspberry (*Rubus strigosus* Michx.) is one of the principal small fruits grown and the plantings aggregate several hundred acres. At least 90 per cent of this acreage is planted to the variety Cuthbert. A few plantings of Marlboro and Herbert make up the remainder.

Three brief accounts (8, 9, 10) have been published and a report (Division of Botany, Annual Report 1921–1922) in more detail is in press; all of which are based on the same work that is summarized below.

SYMPTOMS OF LEAF CURL

Leaf curl is a systemic disease, in that all of the new growth, formed subsequent to infection, is more or less typically affected. The leaflets on the first- and second-year wood are much darker green than normal and the mid-vein arches downward throughout its length. A similar arching of the lateral veins causes a downward curling of the entire margin of the leaflet. Very often a greater tension in the arching of the mid-vein results in the tip rolling under. .Another constant characteristic of the curled leaflets is the "gathering" of the interveinal tissue along the mid-vein and lateral veins. Also the interveinal tissue is often "puckered" and arched between the lateral veins. These results of uneven development are more pronounced when the plants are growing rapidly and the tissues are succulent. When growth is less rapid or the infection of the plant has been recent, the "gathering" along the veins in certain areas may be marked, without much if any resultant curling. Not all cases of slight "gathering or puckering" along the veins, however, are attributable to the leaf curl disease. Very slow growth in the early spring, insect injuries and continued hot dry weather often cause the same effect. In such cases, as soon as these conditions are passed the new growth is normal. In the case of leaf curl all subsequent growth without regard to weather conditions is typically curled. The deformation usually becomes more pronounced as the season advances, until in the case of first-year canes, the tip is stunted and ends in a few small light yellowish green and severely curled leaves. The symptoms of curl are similar for the three varieties, Cuthbert, Marlboro and Herbert. In fact, the leaf characters of these varieties are so masked in the curled plant that it would be difficult to distinguish the variety.

Usually the first-year wood, if infected early in the season does not reach the normal height. If infected in midseason or later, the effect of the disease which results in the dwarfed tip is not reached. In this way curled first-year wood may or may not be dwarfed depending upon the time of infection and the vigor of the plant. The fruiting laterals on a diseased second-year cane are short, upright in habit and the leaves are small, dark green and more or less typically curled. Frequently

the tissue midway between the lateral veins loses its green color and becomes blanched or slightly bronzed. This type of mottling is easily distinguished from that which is characteristic of the mosaic disease. The fruit which develops is small, has very little pulp and is lacking in flavor. In all respects, the effect on the fruit is similar to that produced by mosaic and anthracnose.

Curled leaves are developed in 4 to 6 weeks after infection. All branches or new suckers developed from the stem or roots after curled leaves are formed at the tip, are typically curled. All the leaves developed previous to infection remain normal. The new canes formed the year after infection are much shorter than normal. Individual plants have not been observed for sufficient length of time to enable one to draw definite conclusions, but it is believed that after 3 or 4 years the canes develop only to a height of a few inches and that in this way the disease is finally fatal.

Leaf curl in many respects suggest similarity to potato leaf roll in its effect on the plant. The fundamental foliage symptoms are the same, suggesting inhibition of the translocation of starch, or some disturbance which results in starch accumulation. The staring habit and the general dwarfing effect is common to both diseases. Also both are systemic and the histological studies have shown a necrosis of certain elements of the phloem and pericycle in the raspberry which resembles potato phloem necrosis. Also both are aphid disseminated. Although the two diseases may be alike in gross symptoms, without there being much in common in regard to their cause, it is at least plausible to suspect that raspberry leaf curl and potato leaf roll both belong to the same group of related diseases and that their causes may be similar in nature.

SYMPTOMS OF MOSAIC

Mosaic is noticeable in a plantation from a distance because of the dwarfing of the canes, the sparse yellowish foliage and thin growth. Once mosaic appears in a row of plants it soon spreads, so that for the distance of several meters every cane is affected. The fruiting canes near the middle of the long diseased areas (diseased for more than 3 years) are short and very slender. They may be only little more than half as tall as healthy canes and often less than half the diameter. The laterals developed on these dwarfed canes approach the average length of healthy laterals, but they are spindly. The leaves are not over one-half the size of those on healthy plants. Many of the leaves show the

large, green, blister-like mottling while others show only slight mottling. The leaves have a dull green or even yellowish appearance in contrast with the shiny green of the healthy leaves. The veins are more prominent than normal and appear to be slightly sunken, causing a fine marking of the upper surface. These leaves soon bronze or turn yellow-green in the summer. The suckers from the roots of such canes are usually shorter than the canes of the preceding year and show distinct coarse and fine mottling in all the leaves.

The fruit developed on a cane which shows the dwarfing effect is largely worthless. Much of the fruit on mosaic canes did not develop a pulp during 1921 which was hot and dry at the time the fruit was maturing. That which did develop a pulp was tasteless. Where there was a large percentage of mosaic, the quality of the fruit was greatly impaired even though the dry and seedy berries were not picked.

All the symptoms above described for a plant which has been diseased for probably three years or more are also shown, but often to a less degree, in plants which have been affected only one or two years. Fruiting canes and suckers near the ends of diseased areas are almost as tall and as large in diameter as healthy canes. The leaf symptoms on the fruiting canes are the same as described above except that there is probably less tendency to bronze and turn yellow. In some cases, tall sturdy fruiting canes normal to all appearance, except for the definite leaf mottling, have been noted with mottled suckers which are also normal in height and diameter.

At the ends of the diseased areas there are often found two stages of the invasion into the healthy plants. There are some plants, with one or more of the suckers showing marked mosaic symptoms from the oldest leaf to the tip, and others in which the suckers show no signs of mosaic except in the tip leaves and these may be either finely or coarsely mottled. These two conditions are due to whether infection occurred early in the spring or during the summer.

The most constant and diagnostic symptoms of this disease are shown in the leaves on the suckers. No difference has been noted between those coming from roots diseased for several years and those apparently infected in the spring when they started growth. Before the middle of June the leaves show large irregular green blisters which arch upward. The blisters are not confined to the arrangement of the veins in any way. The tissue between these blisters is lighter green than normal or has a yellowish appearance. Severely blistered leaflets curl downward while the mid-vein remains straight and thus forms a longitudinally rolled

leaflet. In 1921 very high temperature and drought conditions prevailed for about 6 weeks beginning the middle of June. During this time the suckers grew slowly and made short internodes. The leaves were normal except that the leaflets were broader and the tip curled under. A dry corky area was always to be found on the under surface of the mid-vein about one-third of the way from the base of the leaflet. The leaves formed during this period on affected suckers did not show any mottling. During August and September under normal conditions of moisture the suckers made the usual growth with normal foliage. All suckers, which had shown the large blister-like mottling in the spring now developed a fine yellowish speckled mottling, without any rolling of the leaflets. Also recently infected suckers, which showed no mottling in the vernal foliage, developed the fine mottling. In some cases coarse blister-like mottling was produced on some leaves, but this was exceptional.

Many of the Cuthbert suckers affected by mosaic (probably one-fourth) form 1 to 6 or more laterals from the axils of the leaves. These grow into long leafy branches and the axillary buds on them develop fruiting laterals the next year. Such suckers do not grow to normal height.

There is some difference in the appearance of mosaic on the three varieties grown commercially in the Niagara district. In the Cuthbert with its flat, thin, light-green leaf, the mosaic mottling shows very prominently. In the Marlboro with the leaves more or less rolled and fluted due to an arching of the tissue between the lateral veins, the mottling is not distinct, except when abundant and where the yellowish areas are large. The natural dark green of the leaf also seems to mask the finer mottling more than in the Cuthbert. In the Herbert, with leaves similar to the Marlboro as regards color and fluting, the mottling seems to show more prominently than in either of the other two varieties. Even slight mottling results in distinct yellow areas in the dark green leaf and pronounced mottling results in a yellow leaf. The character of dwarfing is shown by all three varieties. More Cuthbert suckers showing pronounced mottling are found which are not markedly dwarfed than in the case of the Marlboro and Herbert. In these two varieties the canes are dwarfed severely and uniformly. The difference in dwarfing is apparently due to a time factor. It is believed that the Marlboro and Herbert respond very quickly after infection and are soon dwarfed. The Cuthbert seems to be slower to respond and usually is not markedly dwarfed until 2 years after infection.

Cases of mixed symptoms of leaf curl and mosaic have been found in the same plant or in the same diseased area in only two instances. The evidence was clear that the plants were affected by both diseases and different parts of the same leaflet often showed the characteristic symptoms of each disease. It is believed that it may not be uncommon to find the two diseases occurring simultaneously. The previous confusion of the symptoms of leaf curl and mosaic in other districts may be due to a high percentage of mixed infection.

ECONOMIC IMPORTANCE

There are too few published statements on which to base estimates of the losses due to leaf curl and mosaic. One or the other or both diseases are probably present in all of the larger small-fruit districts of northern United States and Canada. Melchers (7) and Weiss[1] found leaf curl in Ohio, Michigan, California, Washington, Minnesota and Manitoba. To this range may be added all parts of Ontario and Quebec, Prince Edward Island and Nova Scotia. Leaf curl is also a disease of some importance in Illinois (1) and New Jersey (4). So far as mosaic is concerned there is no definite information concerning its range. It is known to the writers to be present in Nova Scotia, Prince Edward Island, Quebec, Ontario, Michigan, New York and Connecticut. Within this wide range, leaf curl and mosaic have been credited with serious destruction. The "running out" of varieties, especially the Marlboro and Cuthbert and the marked decline in acreage in many districts have been credited to one or the other of these two diseases.

A survey of the conditions in the Niagara Peninsula of Ontario was conducted during the spring of 1921. In this district, which is about 60 miles long, counts were made for the amount of leaf curl in 136 commercial plantings of the variety Cuthbert. While over 10 per cent leaf curl was found in many plantings, the highest average for the different local districts was 6 per cent and the general average was about 4 per cent. In 18 plantings of Marlboro and 27 of Herbert, there was an average of less than 1 per cent leaf curl. The average amount of mosaic in plantings of the variety Cuthbert was about 20 per cent. Plantings of Marlboro showed an average of 27 per cent mosaic, while in the variety Herbert there was only about 1 per cent mosaic.

In the district under discussion leaf curl is enphytotic and although the average loss in stand of 3.8 per cent for 1921 is not large in itself, the cumulative loss over a period of years is an important factor. Most

[1] Weiss, Freeman. Manuscript previously cited.

growers rogue the plants affected by leaf curl in June to prevent the undesirable fruit from being picked. The spaces are usually left unfilled. Roguing in June has had very little if any effect in the control of leaf curl and therefore the percentage of missing plants has increased from year to year. Under the present methods of culture in the district, it is estimated that on the average, a grower has been losing a total of about one year's crop in the life of a plantation or about 8 per cent in gross returns annually.

The losses from mosaic are more difficult to determine. Mosaic has become epiphytotic in the last two years and if it continues, the present indications are that the majority of the plantings of Cuthbert and Marlboro will be destroyed. The practice has been to obtain stock for planting from a neighbor's fruiting plantation. In this way many plantings have been set in the last few years with a high percentage of mosaic plants scattered through them. Plantings made only 3 or 4 years ago have been found with over 30 per cent mosaic as contrasted with plantings at least 10 years old with less than 5 per cent mosaic. It is not uncommon, however, to find in plantings both young and old, numerous areas of mosaic in the rows which are from 3 to 7 meters long. It is evident from the dwarfing that these mosaic areas are often the result of spread in both directions in the row from a single infected plant. The grower has not been accustomed to rogue the plants affected by mosaic and therefore the amount of the disease has increased yearly. The yield of Cuthbert plants affected for 2 years or less is usually not seriously reduced. After 2 years the small number of suckers developed and their dwarfed nature, together with the tendency of the fruit that is set to have very little pulp, leads to a great reduction in yield and to very poor quality. Mosaic is estimated to have reduced the yield from the affected plants in 1921 about 50 per cent. With this estimate as a basis the average loss in gross returns per acre for Cuthberts was about 10 per cent.

SPREAD OF LEAF CURL AND MOSAIC

Both diseases in this district spread only slowly from a diseased plant to neighboring plants. The original roots in continuous rows of plants often are obscure but in general it is true that leaf curl spreads only to the next adjacent plant in a year's time or at most to the next two. Mosaic appears to spread a little faster and an annual spread to the next two plants is common. Newly infected plants appear annually at some distance from old infections. These new areas of infection rarely amount

to more than one-fourth of the number of previous areas. It is thus seen that if the original percentage of either disease is low at time of planting that it takes several years to account for high percentages. The plant affected by mosaic, especially in its earlier stages, is often not recognized by the grower as a diseased plant. It is believed from all the field evidence that the present difference between the enphytotic nature of leaf curl and the epiphytotic nature of mosaic in this district is due largely to the amount of each which has been carried over into the new plantings.

The only plausible and constantly associated agent of inoculation that has been found so far is the aphid, *Aphis rubiphila* Patch. This aphid while never occurring abundantly in this district is commonly found in small scattered colonies. From the writers' observations the following tentative facts have been ascertained regarding the life history of this aphid. The eggs are laid in the fall on the cane in the crevice between it and the buds. The stem-mothers emerge sometime in May, shortly after the fruiting spurs begin their development. The first abundant colonies of young are found on the new suckers late in May and in June, and there is a rapid dispersal at this season. During the summer months, according to weather conditions, the aphids either practically disappear or exist in small colonies in a more or less stationary position. There is practically no movement on the plant or to other plants during the warm weather. In the autumn, cooler weather and frequent rains lead to another season of rapid multiplication and dispersal. So far as known, no winged stage is developed and there is no migration to other food plants. Counts made in the early spring have shown that the sucker affected by mosaic due probably to its more succulent foliage is a more favorable food plant for the development of colonies than is the healthy sucker. The sucker affected by leaf curl is also more favored by the aphids than is the healthy sucker but to a lesser degree than that affected with mosaic. In this way the character of the newly affected suckers enhances the chances of dissemination by harboring larger and more active colonies of aphids than do the healthy plants.

Many different ways of artificially inoculating several hundred healthy plants with the causative agents of leaf curl and mosaic have all failed. Provision for developing selected strains of aphids were made in 1921 but the work of transferring the aphids to healthy plants was only begun just previous to the severe drought which practically eliminated the aphids and made further work impossible. In six instances leaf curl

was successfully transmitted by transferring aphids under conditions where other factors were eliminated. The first set of transfers for mosaic transmission, however, were made at the beginning of continued hot weather and no results were obtained. The field evidence is so conclusive that there is little doubt that both diseases are transmitted by *Aphis rubiphila*.[1]

VARIETAL SUSCEPTIBILITY

Plants of the following red and purple varieties have been seen affected by mosaic: Abundance, Brighton, Brilliant, Columbian, Count, Cuthbert, Dr. Reider, Eaton, Empire, Golden Queen, Haymaker, Herbert, Highland Hardy, Idaho, June, King, Louboro, Marlative, Marlboro, Marldon, Minnesota, Newman No. 1, Newman No. 23, Newman No. 24, Ontario, Royal Purple, Ruby, Segrist, St. Regis. The data were obtained largely from an examination of the horticultural plots of the Central Experimental Farm, Ottawa, Canada, and of the New York Agricultural Experiment Station, Geneva, New York. A mosaic, similar in all gross symptoms at least, is common on some cultivated varieties of black raspberries.

According to Weiss[2] leaf curl is a common disease of the wild *Rubus strigosus* Michx. in Minnesota and in Manitoba as far north as the upper end of Lake Winnipeg. It has also been found common on the same host in northern Ontario. The variety Cuthbert is very susceptible but the Marlboro and Herbert are rarely affected. In one instance an unknown variety of black raspberry was found with typical leaf curl. Also 3 of 5 ornamental wineberry bushes (*Rubus phoenicolasius* Maxim.) were found affected by leaf curl.

CONTROL MEASURES

The first and most important factor in the control of either leaf curl or mosaic would seem to be an attempt to produce healthy planting stock for local use. Present indications are that stock in small isolated plots can be kept healthy for transplanting without much difficulty. The most of the increase in the fruiting plantation is from plant to plant. New areas are most easily accounted for by the dragging of diseased canes during the cultivating and pruning operations, or by the occasional

[1] Prof. B. T. Dickson, of Macdonald College (Quebec) who has been working on several mosaic diseases, reported verbally during the discussion on raspberry mosaic at the Toronto meetings that he had successfully transmitted this disease in three instances by transferring *Aphis rubiphila* to healthy plants.

[2] Weiss, Freeman. Manuscript previously cited.

transfer of the aphids themselves by strong winds. It is not believed that a plantation set with healthy stock will be subjected to much chance of infection, if it is separated from other plantings by a distance of as much as 30 meters. These statements are borne out by general observation where high percentages are clearly due to a large number of old diseased areas and by many old plantings which show very few such areas. In the case that in some districts the disseminating agent develops a winged stage or has other hosts, the above arguments would have but little weight.

In young plantings where the amount of either disease is not too great, control may be possible by roguing at a season when the aphids are more or less stationary. In this district leaf curl is easily distinguishable shortly after the growth begins in the spring and before the aphid eggs hatch. Present experimental control measures for leaf curl are based on removing the affected plants during this interval. The symptoms of mosaic are not distinguishable in the spring until after the aphids are dispersed in large numbers. It is believed, however, that normally the aphids are stationary enough in mid-summer to insure that all affected plants have had a chance to develop symptoms since infection. Experimental control for mosaic by roguing has been limited to an indefinite period, beginning about two weeks after continuously warm summer weather has begun. It is true that in excessively hot weather the recently infected plants do not show the symptoms of mosaic, a condition which interferes with successful roguing.

No attempts apparently have been made to develop a desirable commercial variety either by selection or by breeding which is resistant to either of these diseases. For diseases of this type it would be fortunate if resistant varieties could be produced since the propagation of disease free stock and constant roguing in the hands of the grower are very unsatisfactory measures to depend upon.

DOMINION LABORATORY OF PLANT PATHOLOGY,
ST. CATHARINES, ONTARIO, CANADA.

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FASCIATION AND PROLEPSIS DUE TO CROWN GALL

ERWIN F. SMITH

WITH PLATES XVI TO XX

Several times in recent years (*Science*, n. s., 1916, Vol. 44, p. 781; *Jour. of Agric. Res.*, 1916, Vol. 6, Pl. XVIII; *Introduction to Bacterial Diseases of Plants*, 1920, text and figs. 335, 340) the writer has called attention to the fact that fasciation of shoots may be one of the results of crown-gall inoculations, but as these remarks were incidental in writings devoted to various phases of crown-gall research not much attention appears to have been paid to them. I propose, therefore, to call attention to the subject once more directly and to show an additional example.

The fusion and flattening of various organs known as fasciation is very common in a great variety of plants and, being often so striking as to cause great wonder, many good pictures have been published in books and papers on plant Teratology. A similar phenomenon occurs in the animal world and there are many interesting figures of trunk and limb duplications or fusions, especially of parts of the human body, in treatises on "Monsters." Recently Stockard and others have attacked the problem experimentally.

There have been many speculations among botanists as to the cause of these abnormalities, but thus far very few enlightening comments.

The cause of fasciation is a problem to be approached and solved by experimental methods, rather than by observation, and the following remarks may be considered in the light of suggestions as to the direction of approach in individual cases rather than as a key to the whole situation.

I have demonstrated that fasciation can be produced experimentally by inoculating *Bacterium tumefaciens* Sm. and T. into the leaf axils of a variety of plants (Nicotiana, Pelargonium, Ricinus, Brassica, Tropaeolum) and the immediate presumption is that many other fasciations are disturbances due to the penetration of foreign organisms into the growing point in such a way as to crowd and divide it or to bring about irritation leading to fusions.

Each particular fasciation is a problem by itself and I would not be understood as contending either that all fasciations are due to one and the same microorganism or that all are due to parasites and their products; but only that the hypothesis of a symbiont or of a feeble parasite

(bacterial, fungous or other) offers a hopeful method of attack in individual cases.

In any event, the initial disturbance may be assumed to have begun in the embryonic or dormant bud stage, and in attempting to culture out an assumed parasite one should begin, I think, at the base of the fasciation as the part most likely to contain it. This conclusion is based, in part, on our work on hairy-root in which it was shown (U. S. Department Agriculture, 1911, Bureau of Plant Industry Bulletin 213, p. 101) that the organism occurs in the flat base from which the tuft of roots arises but not in the roots themselves; in part, on recent observations that the crown-gall tissue occupies only the base in such shoots as are described in this note. This absence of the causal organism in the greater part of the fasciation would also serve to explain why examinations of fasciated shoots have always proved sterile.

The first three figures shown (Pls. XVI, A, B, and XVII) are of a nasturtium plant (*Tropaeolum majus* L.) inoculated when young in a leaf axil with *Bacterium tumefaciens* Sm. and T. by means of needle pricks and photographed a month later when the only secondary shoot on the plant was the fasciated one from the inoculated axil. At the time the inoculation was made there was, of course, no shoot in this axil nor even any visible bud. The fasciated shoot developed from a dormant bud along with the growth of the tumor and in consequence of its growth. In other words, the development of the shoot as related to the tumor is not merely a *post hoc* phenomenon, but one actually due to the presence of the tumor. Plate XVI *A* shows the whole plant about ⅓ natural size with the fasciated shoot at its base and all other leaf axils free from shoots. Plate XVI *B* and plate XVII show respectively the lower and upper one-half of the fasciated shoot, enlarged × 5 for the sake of details. In six leaf axils on this fasciated shoot there was an abnormal pushing of buds just as I showed recently for tumor-stimulated shoots on *Bryophyllum calycinum* (Effect of Crown Gall Inoculations on Bryophyllum. *Jour. of Agric. Res.*, Vol. XXI, July 15, 1921, plates 101 and 107).

At the time this fasciated shoot was photographed and fixed for sections there was no evidence of tumor tissue anywhere on its surface, but after fixing in Carnoy's fluid (¼ glacial acetic acid, ¾ absolute alcohol) the flattened shoot became very clear except for internal yellowish areas in the lower one centimeter which were assumed to be due to crown-gall proliferations in the vascular region and shown to be such in stained sections. This proliferating (invasive) tissue is present in

ternally at the base of the shoot, as far up as the arrow, but not in the tissues midway or farther up the shoot. The effect, however, of tumor proximity was a general stimulus of the whole shoot including its dormant axillary buds. The primary tumor is visible in plate XVI *B* at *T*, *T*, with the shriveled base of the subtending petiole at *P*, and below this in the main axis the tumor has extended downward a short distance and burst through the cortex as indicated at *S*. There are also clusters of incipient roots (R). In the upper part, also, (Pl. XVII) axillary buds are pushing at X, X, X and from the axil of L. At *A* and *B* there are two distinct axes of growth. There is a third axis of growth (fasciation) on plate XVI *B* at *F*. The contrast between the size of the main axis *M* and of the fasciated shoot indicates clearly a strong movement of water and elaborated foods into the tumor and the shoot complex.

The plant was inoculated when it was a month old and the photographs were made a month later, *i. e.*, while the plant was still young.

Fifteen plants were inoculated and only one gave a fasciated shoot. All depends, apparently, on whether the needle hits or misses the dormant bud. Most of the other plants, however, developed a shoot, often a strong one, from the dormant bud in the inoculated axil *and from that bud only*, showing very strikingly the effect of the tumor stimulus.

A photograph of one of these non-fasciated tumor-stimulated shoots made on January 14, *i. e.*, two months after the inoculation, is shown on plate XVIII at *2*, the subtending petiole P being now dead.

In the paper on Bryophyllum, I called attention to a certain resemblance of the stimulated shoots to the witch-brooms of "peach yellows," our most destructive peach disease, the cause of which remains unknown, but in which we know that the tree has difficulty in translocating its starch. In that disease the summer sprouts which appear on the trunk and main limbs develop feeble branches in their leaf axils the same season, and so on, until within a space of 5 or 6 months, say from June to November in the latitude of Delaware, 3 sets of weak branches may develop from leaf axils on such shoots, all or most of these shoots dying the following season, at least at the top. It would seem that the proximate cause of the two diseases must be the same, to wit, stimulus of excessive amounts of water and foodstuffs acting locally as the result of phloem injury.

In Bryophyllum, tertiary shoots developed within a few weeks from the leaf axils of the crown-gall stimulated, hasty, secondary shoots. In the nasturtium plant shown in plate XVIII, this process has been carried

one step farther, as far or almost as far as it ever goes in peach yellows, *i. e.*, the inoculated leaf axil of the primary shoot (here designated as 1) has given rise to a secondary shoot (2), this in turn has given rise (always from leaf axils) to two well developed tertiary shoots (3, 3), and these in turn have given rise to four quaternary shoots, all within a period of two months.

It is a remarkable thing that within so short a time a crown-gall inoculation should have given rise to 3 sets of shoots and it can be explained only on the supposition that the growth of the tumor caused a powerful centripetal movement of water and foodstuffs which also acted on the adjacent dormant bud (mother of 2) and continued to act excessively on the shoot which developed from it. This is shown also by the fact that the tertiary shoots (3, 3) are twice the diameter of the main axis (1). The terminal leaves on the shoots 2 and 3, 3, were wilted when the plant was brought in from the hothouse for photographing and as the plants were free from insects and otherwise under good conditions, and as all the checks remained normal, I had no way of explaining this except to suppose that the shoots, excessively nourished at first, were finally unable to get enough water through their vascular system, owing to its invasion or compression by the axillary tumor. This is the usual course of events and shoots growing out of crown galls generally die early. Cross sections in series were afterwards made from the base of branch 2 and this hypothesis was confirmed. Very likely the downward movement of elaborated food stuffs (sugar and proteids) in this shoot (2) was also eventually interfered with by the growth of the tumor and that too considerably earlier than the shutting off of the water supply, so that the tumor has acted as a stimulus to the growth of the shoot in both a positive and a negative way: positive by attracting food to itself some of which was also used by the shoot; negative by interfering, through gradually increasing compression, with the normal nightly downward movement of elaborated carbohydrates.

Shoot No. 2 (Pl. XVIII) was not tested for starch because I did not then have it in mind, but since the preceding paragraph was written, I have found (April 13–14) 3 nasturtium plants of a later inoculation (Jan. 20 1922), which show the same phenomenon, *i. e.*, shoots pushing from the axil bearing the tumor and a marked thickening of these shoots some distance away from the tumor *i. e.*, to more than twice the diameter of the main axis, as in Pl. XVIII, 3, 3, with wilting of terminal leaves and in each instance thin cross-sections of the base of the shoots treated with iodine showed in the cortex, pith and medullary rays a marked accumu-

lation of starch, very much more than in the main axis. Here, clearly, the constriction of the base of the shoot by interfering with the normal downward movement of the elaborated foodstuffs was the cause of the thickening of its axis and of the pushing of its dormant buds, and further constriction caused the wilting. The location of the enlargment of the shootaxis at some distance from the tumor shows also that for some time, in each case, there was no compression of the brse of the shoot. In one of these plants a branch corresponding to 4 of plate XVIII had a so pushed buds, making four sets of shoots in less than three months.

In plate XIX, made from a freehand section treated with iodine, may be seen the abnormal storage of starch in the cortex of one of the swollen axillary shoots referred to in the preceding paragraph. The constricting tumor was about one centimeter below this point. Plate XX is from the pith of another plant in the same series. Here also the shoot was constricted by the tumor and the downward movement of sugar and proteids was interfered with.

In this disease, therefore, we may have all varieties of stimulating secondary effects on normal tissues from prolepsis of uninjured leaf and flower buds and root anlage located in the vicinity of the tumors, through simple fusions or divisions (fasciations), to the breaking up of the dormant bud, or of a cambium, into dozens and even hundreds of small vegetative fragments which may either grow as roots or shoots on the surface of the tumor or be buried in its depths. Due to the same organism we may have, therefore, both organoid and histioid galls although these are at the two poles of Küster's classification. The tumor, of course, also exerts purely destructive effects on surrounding tissues but these do not concern us here.

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DESCRIPTION OF PLATES XVI TO XX

PLATE XVI. *A*. Hop strain of crown-gall organism (Sunflower Colony 1) inoculated November 17, 1921, in a lower leaf axil of *Tropaeolum majus* L., by means of needle pricks. Result: A small tumor, not clearly visible here, above which is a fasciated shoot—the only secondary shoot on the plant. Photographed December 19, 1921. About one-third natural size. Actual size of the twisted, flattened shoot, 1¾ inches by ¼ by 1/6 inch, the last two measurements being in the middle part.

PLATE XVI. *B*. Base of the fasciated shoot. Stub of the subtending petiole (now

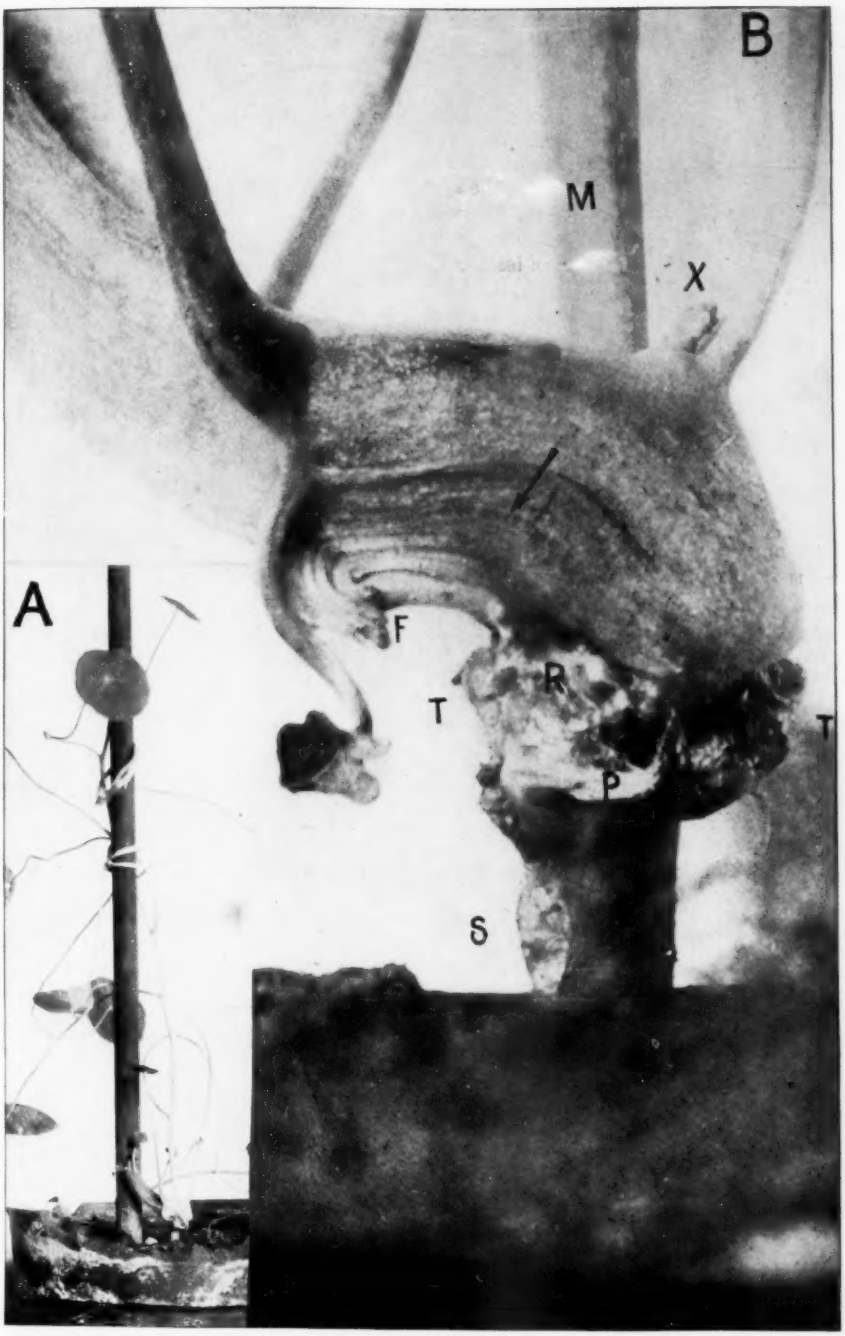
dead) at *P*. Primary tumor at *T*, *T*. A downward extension of this tumor has burst through the cortex of the main axis (M) at *S*. A shoot is pushing in the leaf axil *X*, and another was pushing from the axil of a dwarfed leaf on the back side of the base. There are incipient roots at *R*. At *F* there is a third secondary (fasciated) axis of growth, the other two being at the apex of the shoot (Pl. XVII). On sectioning, the tumor tissue was found to have extended upward in the vascular region of the shoot as far as indicated by the arrow. × 5.

PLATE XVII. Upper part of fasciation on plate XVI. B, showing two axes of growth, *A*, *B*. This part is quite flat. Main axis of the plant at *M* with a petiole *p* at its right. Buds were pushing from three visible leaf axils *X*,*X*,*X*, and also on the back side from the axil of leaf *L*. × 5.

PLATE XVIII. Inoculated axillary crown gall on nasturtium: 1, main axis unbranched except as shown in the photograph. *P*, petiole in the axil of which the plant was inoculated by needle pricks November 17, 1921, *t*, tumor that developed; *P* 2, secondary shoot derived from an uninjured axillary bud growing as a result of the tumor stimulus; 3, tertiary shoots, very plump; 4, quaternary shoots. Leaves wilting from late interference with water-supply. Photographed January 14, 1922. ¾ natural size.

Pl. XIX. Starch in cortex in a swollen axillary nasturtium shoot collected in the morning. Trans-location blocked by tumor in same axil. The whole cortex was like this.

Pl. XX. Starch in pith in a swollen axillary nasturtium shoot collected in the morning. Translocation blocked by tumor in the same axil. The whole pith was like this; also many of the medullary rays. Not the same plant as that shown in Plate XIX.



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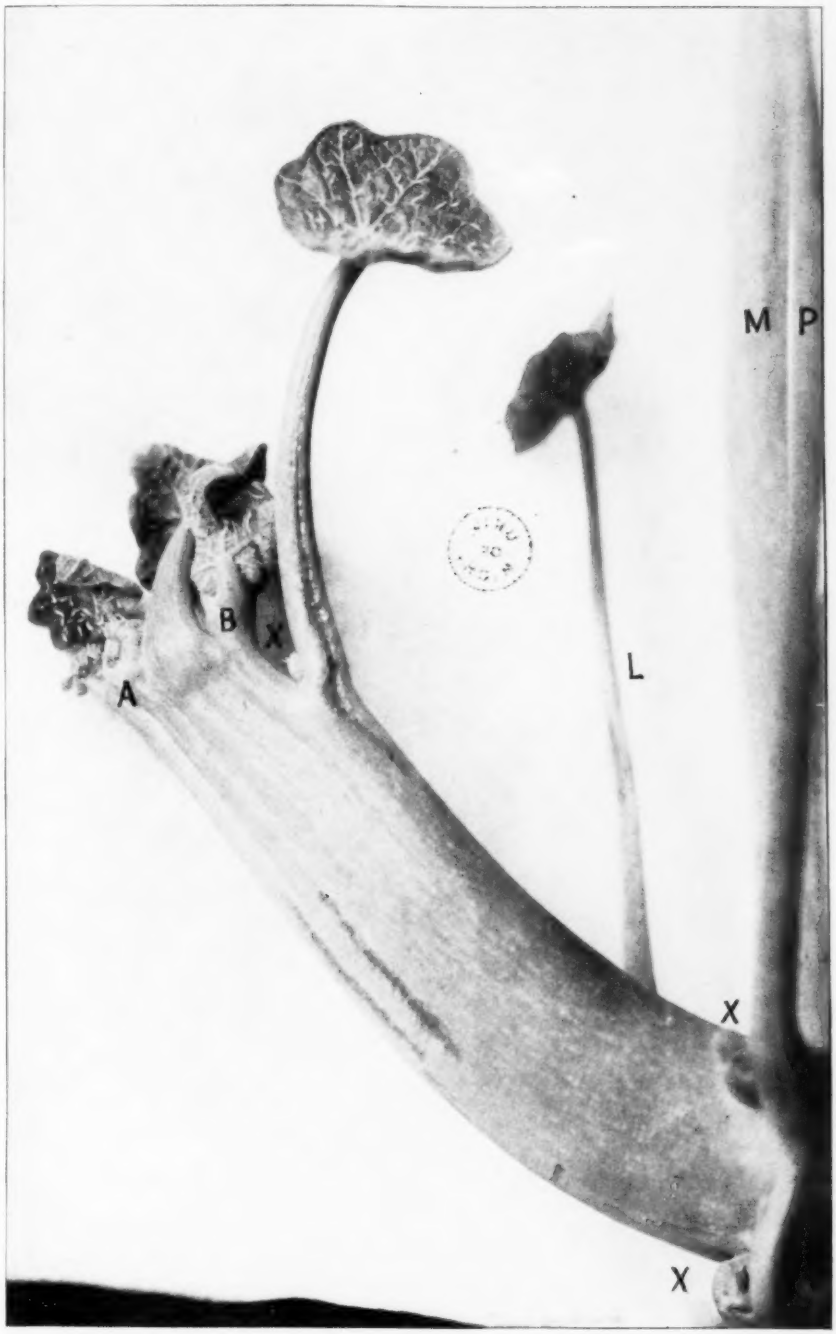

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PATHOGENICITY OF THE OLIVE KNOT ORGANISM ON HOSTS RELATED TO THE OLIVE.[1]

CLAYTON O. SMITH

WITH PLATES XXI AND XXII

INTRODUCTION

The bacterial organism *Pseudomonas savastanoi* E. F. S., has been carefully studied by different investigators and its pathogenicity on the olive fully determined. Its pathogenicity, however, on other closely related hosts has not been definitely proved. Savastano[2] on inoculating *Nerium oleander*, *Osmanthus* (Olea) *fragrans*, grapes, peaches, plums, almonds, figs, apples and pears, with pure cultures of the olive-knot organism as well as with olive-knot tissue obtained negative results. Smith[3] also reports negative results in his inoculations on the oleander, *Chrysanthemum frutescens*, several species of Fraxinus, privets and other plants more or less closely related to the olive. On one host, *Fraxinus ornus*, slight swellings were visible, but the experiment was terminated by an accident before the results were fully determined.

PLAN OF EXPERIMENT

The experiments on which this paper is based extended through the years 1919 to 1921 in the course of which plants more or less closely related taxonomically to the olive were inoculated. Pure cultures of *Pseudomonas savastanoi* isolated from the typical olive knot and incubated at 25° C. for from 24–48 hours were used. Ten inoculations or multiples of ten with control punctures were made at the same time on each host. The olive was included in order to check the virulence of the culture used. A steel needle was employed in the inoculation work and no protection was given the punctures after they were made. The data were taken at the close of the growing season usually in December of the year the experiment was made.

The following species were inoculated: olive (*Olea europaea* Linn.), *Fraxinus velutina* Torr., *F. floribunda* (S. P. I. 47687) which closely

[1] Paper No. 88. University of California Graduate School of Tropical Agriculture and Citrus Experiment Station, Riverside, California.

[2] Savastano, L. Sulla trasmissibilità del bacillo della tubercolosi dell' olivo nell' oleandro. Bol. Arbor. Ital., **4**: 86–87. 1908.

[3] Smith, Erwin F. Recent studies of the olive-tubercle organism. U. S. Dept. Agr. Bur. Plant Industry Bul. 131, part IV, p. 25–43. 1908.

resembles *F. Ornus* Linn., *Adelia* (Forestiera) *acuminata* Michx., privet (*Ligustrum ovalifolium* Hassk.), *Chionanthus virginica* Linn., *Osmanthus fragrans* Lour., *Osmanthus* (Olea) *aquifolium* Sieb., Vinca, *Thevetia nereifolia* Juss., *Nerium oleander* Linn., *Coprosma baueri* Endl., *Carissa grandiflora* DC., *Chrysanthemum frutescens* Linn., *Elaeagnus angustifolia* Linn., lilac, and jasmine (probably *Jasminum primulinum* Hensl.) and several species of Prunus that have proved especially susceptible to *Pseudomonas tumefaciens* Sm. and T.

RESULTS

The tests for 1919 were doubtful except on Adelia and privet. Tests made the following two years proved other hosts to be susceptible, as shown by typical knots and less evident response in the form of definite lesions. The various hosts reacted somewhat differently. On some of the hosts the results were negative; on others only slight hypertrophies developed at the margin of the wound, which in some cases eventually gave rise to a definite canker of some size. On still other hosts as illustrated in plate XXI subspherical knots were produced closely resembling those of the olive. These results will be considered more fully under each of the hosts. Of the hosts mentioned above the following gave negative or doubtful results: *Osmanthus fragrans*, Vinca, *Thevetia nereifolia*, *Nerium oleander*, *Coprosma baueri*, *Carissa grandiflora*, *Chrysanthemum frutescens*, *Elaeagnus angustifolia*, lilac, and species of Prunus. Privet gave negative results except in the tests of 1919, where small knob-like growths developed, (Pl. XXI, fig. 16). *Osmanthus aquifolium* and *Chionanthus virginica* gave definite lesions. The hosts which gave knots or galls were *Fraxinus velutina*, *F. floribunda*, *Adelia acuminata*, *Jasminum primulinum*.

Adelia acuminata (Tab. I) developed typical knots that closely re-

TABLE 1

Inoculations on Adelia acuminata

Date	Punctures	Number[1]	Diameter in mm.
June 28, 1919	10	6	5–15
Aug. 4, 1919	10	8	2–4
Aug. 12, 1919	10	6	2–3
Apr. 15, 1920	10	6	10–15
Apr. 23, 1920	10	6	3–8
June 15, 1920	10	9	4–8
June 21, 1920	10	7	1–2
July 16, 1920	10	8	2–3

[1] In Tables 1 to 5 inclusive this column indicates the number of hypertrophies which developed.

sembled those of the organism on olive, but were somewhat smaller, being from 2–15 mm. in diameter (Pl. XXI, figs. 13–14). They were somewhat irregular in shape and protruded from the normal tissue as hypertrophies. Section of the knots showed definite bacterial cavities containing numerous bacteria. This host is closely related to the olive and has been reported to have been successfully grafted to it.

Fraxinus velutina and *F. floribunda* (the latter a small plant sent from the United States Department of Agriculture, Bureau of Seed and Plant Introduction as number 47687) were tested (Tab. 2). The latter species is native to India and much resembles *F. Ornus*, the species used by Dr. Erwin Smith in his experiment, where small enlargements developed about the punctures.

TABLE 2

Inoculations on Fraxinus

Date	Species	Punctures	Number	Diameter in mm.
Mar. 4, 1920	Velutina	10	6	4–8
Mar. 11, 1921	"	20	6	2–11
Mar. 21, 1921	"	30	18	2–5
Mar. 21, 1921	"	10 check	0	
Apr. 13, 1921	"	10	9	4–5
Apr. 13, 1921	"	10 check	0	4–5
May 5, 1921	Floribunda	5	5	4–10

In both of the above species galls were formed at punctures in the young succulent stem and on the petioles of leaves (Pl. XXI, fig. 2). The knots of *F. velutina* were still fresh and green a year after the inoculation (Pl. XXI, fig. 1). These have made but little new growth during the second season, but pointlike elevations have appeared that in some instances extend 2 mm. beyond the general surface of the knot. These knots (Pl. XXI, fig. 3–4) seem to form readily upon inoculation of actively growing tissue. The inoculations made early in the season were the best developed while those made late in the season (after June) were generally unsuccessful.

Chionanthus virginica (Tab. 3) when inoculated with the olive knot organism did not form typical knots except on the leaves (Pl. XXI, figs. 10–11), but it did produce definite lesions (Pl. XXI, figs. 8–9). The first stages showed a water-soaked, brownish area above and below the puncture. In two weeks a slight swelling developed around the punctures (Pl. XXI, fig. 12). This hypertrophied tissue was of a lighter green color than normal tissue. At this time the original puncture had developed into an enlarged depressed spot, caused by a slight killing of the tissue.

TABLE 3

Inoculation on Chionanthus virginica

Date	Punctures	Number[1]	Diameter in mm.
Apr. 10, 1920	10	6	3–7
Apr. 30, 1920	20	11	4–15
June 15, 1920	20	15	4–15
July 16, 1920	20	11	3–12
July 26, 1920	20	13	3–25
Apr. 13, 1921	20	18	10–15
Apr. 13, 1921	10 check	0	
Apr. 29, 1921	10	3	5–13

Definite scars or lesions eventually developed at the point of inoculation, with hypertrophy of tissue at their margins. These lesions continued to increase in size with the growth of the host. Their surfaces were covered with a dark hypertrophied growth with numerous fissures. Inoculation of young tissue frequently resulted in a modification of its growth by causing a shortening and thickening of the shoot (Pl. XXI, fig. 9). The abnormal hypertrophies, however, developed more often within the normal limits of the tissue than as an external knot. Inoculation in the veins and petioles of the leaf showed positive results in lesions in the twisting of the leaf and at length in the production of small galls (Pl. XXI, fig. 10).

Osmanthus aquifolium when inoculated with the olive knot organism (Tab. 4) did not develop any large external knots. The enlargement of tissue generally involved the regions immediately surrounding the puncture (Pl. XXI, fig. 6). The healing tissue at the margin of the puncture sometimes rounded up in an abnormal manner as compared with control punctures. In some instances very small (3–4 mm.) round shaped galls developed (Pl. XXI, fig. 7). Their growth did not continue, however, and was only slightly suggestive of the initial olive knot. The tissue that developed about the punctures was of a darker color and rougher than normal callous tissue. The olive knot organism was successfully cultured from the hypertrophied tissue of this knot (Pl. XXI,

TABLE 4

Inoculations on Osmanthus aquifolium

Date	Puncture	Number	Diameter in mm.
June 18, 1920	20	10	4–8
June 21, 1920	20	12	3–6
July 16, 1920	10	5	6–15
May 16, 1921	30	16	3–5

[1] The hypertrophies that here develop were more in the nature of lesions than knots.

fig. 7) 6 months after inoculation. The hypertrophied tissue at length dies becoming a dark color and very hard and dry.

More recent inoculations have given more typical knots (Pl. XXI, fig. 5) which were more like those produced in Adelia and Fraxinus.

On *Jasminum primulinum* (Tab. 5) definite and characteristic knots developed (Pl. XXI, fig. 15). These first began to form around punctures at the margin of the wounds as aerial hypertrophies which were at first of a greenish color but later became hard and dried and of a darker color. Little hypertrophy if any took place within the normal tissue although some darkening of tissue could be observed in the pith (Pl. XXII, fig. 19).

TABLE 5

Inoculations on Jasminum primulinum

Date	Punctures	Number	Diameter in mm.
Jan. 31, 1921	20	1	2
Feb. 21, 1921	10	7	3–5
Nov. 11, 1921	20	7	3–5
May 16, 1921	10	9	3–5
June 27, 1921	20	18	3–9
July 18, 1921	10	8	2–5
July 29, 1921	10	7	2–7

REISOLATION OF ORGANISM

The causal organism isolated from the artificial knots on the various hosts was then used for inoculation on the olive (Tab. 6.) Reisolations were made from Jasminum, Fraxinus, Olive, Chionanthus, Adelia and Osmanthus. When these organisms were inoculated into the olive, typical olive galls developed except with Fraxinus. The organisms isolated appeared to resemble the olive-knot organism on glucose potato agar and other media.

PATHOLOGICAL HISTOLOGY

The olive-knot organism is characterized by the production of masses of bacteria in definite cavities within the hypertrophied tissue of the olive. This is a very evident difference from galls of *Pseudomonas tumefaciens* on various hosts where very few organisms are present within the hypertrophied cells.

Microtome sections of the knots of the various hosts giving positive results with *Pseudomonas savastanoi* were examined microscopically. These were cut with a sliding microtome after being fixed in Carnoy's solution. They were stained with acid fuchsin. In general the sec-

TABLE 6

Reisolation of Pseudomonas savastanoi

Date of Inoc.	Host inoculated	Date of reisolation	Result of reinoculation of olive	
			Number[1]	Size (mm.)
June 9, 1920.....	Chionanthus	Dec. 27, 1920	(20)19	5–10
June 18, 1920.....	Osmanthus	Dec. 28, 1920	(20)15	2–5
July 16, 1920.....	Adelia	Dec. 28, 1920	(20)15	3–12
June 21, 1920.....	Olive	Feb. 15, 1921	(20)19	5–10
Mar. 21, 1921.....	Fraxinus	July 18, 1921	(40)0	
Apr. 13, 1921.....	Chionanthus	May 15, 1921	(40)27	5–25
May 16, 1921.....	Osmanthus	Aug. 16, 1921	(20)15	2–3
May 27, 1921.....	Chionanthus	Aug. 16, 1921	(20)20	2–12
June 27, 1921.....	Jasminum	Aug. 20, 1921	(10)8	3–8

tions of these knots from different hosts showed the same characteristics as the typical olive knot. The organism occurs in mass and is easily demonstrated microscopically. These masses are usually associated with tissue that gives a more brownish stain than the rest. Sections of the knot on the midvein of Chionanthus (Pl. XXII, figs. 17–18) in the center of which was a large cavity, were studied. The presence of bacteria was readily demonstrated near the margin where they had not been washed away by the process of staining the sections.

TABLE 7

Results of the inoculation of different hosts with Pseudomonas savastanoi. The number in parenthesis is the number of knots that developed from 10 punctures. The other numbers give the size of the knots in millimeters

Date	Olive	Fraxinus	Adelia	Chionanthus	Osmanthus	Privet	Jasmine
June 28, 1919	(10)8–20	—	(6)5–15	—	—	(9)2–5	
Aug. 4, 1919	—	—	(8)2–3	—	—	(2)2–3	
Apr. 30, 1920	(4)12–20	—	(6)10–15	0	—	0	
June 15, 1920	(10)8–10	0	(9)4–8	(9)8–10	—		
June 21, 1920	(10)6–10	0	(7)1–2	(6)4–6	—		(1)2
Jan. 31, 1921	(5)6–10	—	—	—	—		(7)3–5
Mar. 11, 1921	(5)6–10	(3)48	—	—	—		—
Mar. 21, 1921	(4)2–5	(9)2–11	—	—	—		—
Apr. 13, 1921	—	(9)4–5	—	(8)10–15	—		—
May 5, 1921	(6)2–5	(5)[2]4–10	—	—	—		
June 27, 1921	(7)3–12	—	—	(9)3–5	(18)[3]3–9		
July 29, 1921	(10)6–10	—	—	—	(7)2–7		

[1] The number in parenthesis denotes the number of puncture inoculations, the one outside the parenthesis the number of knots that developed.

[2] This host is *Fraxinus floribunda*, all others *F. velutina*.

[3] In this test 20 punctures were made.

Sections of *Jasminum primulinum* still showed the old needle puncture through the stem, the path of the needle being filled with hypertrophied tissue which is especially noticeable in the pith and in the xylem region (Pl. XXII, fig. 19).

In certain parts of this needle path can be found numerous organisms. Masses of bacteria were also found in the hypertrophied tissue, especially near those parts that take the reddish-brown stain. The hypertrophy in Jasmine was parenchymatous in nature, but in these knots, vascular elements were often present although usually displaced from their normal position.

SUMMARY AND DISCUSSION

1. Typical artificial knots have been produced with pure cultures of the olive-knot organism on Adelia and two species of Fraxinus. These more or less closely resemble the knot produced on the olive by the same organism.

2. The inoculations on Chionanthus and Osmanthus do not usually result in the production of galls or knots, yet there is a very positive pathological effect, especially a stimulation of the growth of tissue followed often by a slight necrosis.

3. The size of the hypertrophies (knots) produced on the hosts mentioned in 1 and 2 were smaller than those on the olive, with the possible exception of some on *Fraxinus floribunda*. They apparently reach their maximum size in 3 or 4 months after which the tissue gradually dies. This seems to take place sooner than in the knot on the olive.

4. The olive-knot organism has been regarded as parasitic only on the olive. These experiments suggest that it may not be so restricted, but that it may possibly occur naturally elsewhere, especially on certain species of Fraxinus.

5. The organism in no case was infectious in hosts not related taxonomically to the olive. It seems to be restricted to plants closely related botanically to the olive, and especially to those of the family Oleaceae.

6. The limited pathogenicity of the olive-knot organism would seem to separate it definitely from *Pseudomonas tumefaciens*.

DESCRIPTION OF PLATES

PLATE XXI. Results from artificial inoculation with *Pseudomonas savastanoi* on hosts related to the olive.

FIGS. 1–4. Fraxinus. (1) *F. velutina*, inoculated May 4, photographed Dec. 1

1920;[1] (2) *F. floribunda*, two puncture inoculations on stem and two on leaf petioles, May 5, Aug. 5, 1921; (3) *F. velutina* Mar. 21, July 1, 1921; (4) Mar. 11, *F. velutina* Nov. 1, 1920.

FIGS. 5–7. *Osmanthus aquifolium*. (5), Inoculation with olive-knot tissue, July 1, 1921, Jan. 1, 1922; (6), 4 positive puncture inoculations on stem and 7 on leaves, May 16, Aug. 16, 1921; (7) 3 puncture inoculations made on same host, at same time and from same culture as figure 6.

FIGS. 8–12. *Chionanthus virginica*. (8) Advanced cankers such as develop from artificial inoculations on older shoots, June 12, Dec. 12, 1921; (9) results in rapidly growing succulent tissue, from inoculation, July 15, Sept. 15, 1920; (10)–(11), puncture inoculations of leaf parenchyma and midvein, May 26, Nov. 1, 1921; (12), lesions in their early stages of development, showing the abnormally thickened margin, Apr. 3, May 24, 1921.

FIGS. 13–14. *Adelia* (*Forestiera*) *acuminata*. (13), 3 knots on stem, June 30, Dec. 1, 1921; (14), nearly spherical knots, Apr. 15, Dec. 15, 1920.

FIG. 15. *Jasminum primulinum* puncture inoculated, June 27, Dec. 1, 1921.

FIG. 16. *Ligustrum ovalifolium* (Privet). Very small hypertrophies or knob-like growth, the only ones produced, although other inoculations were made at various times, June 28, Dec. 1, 1919.

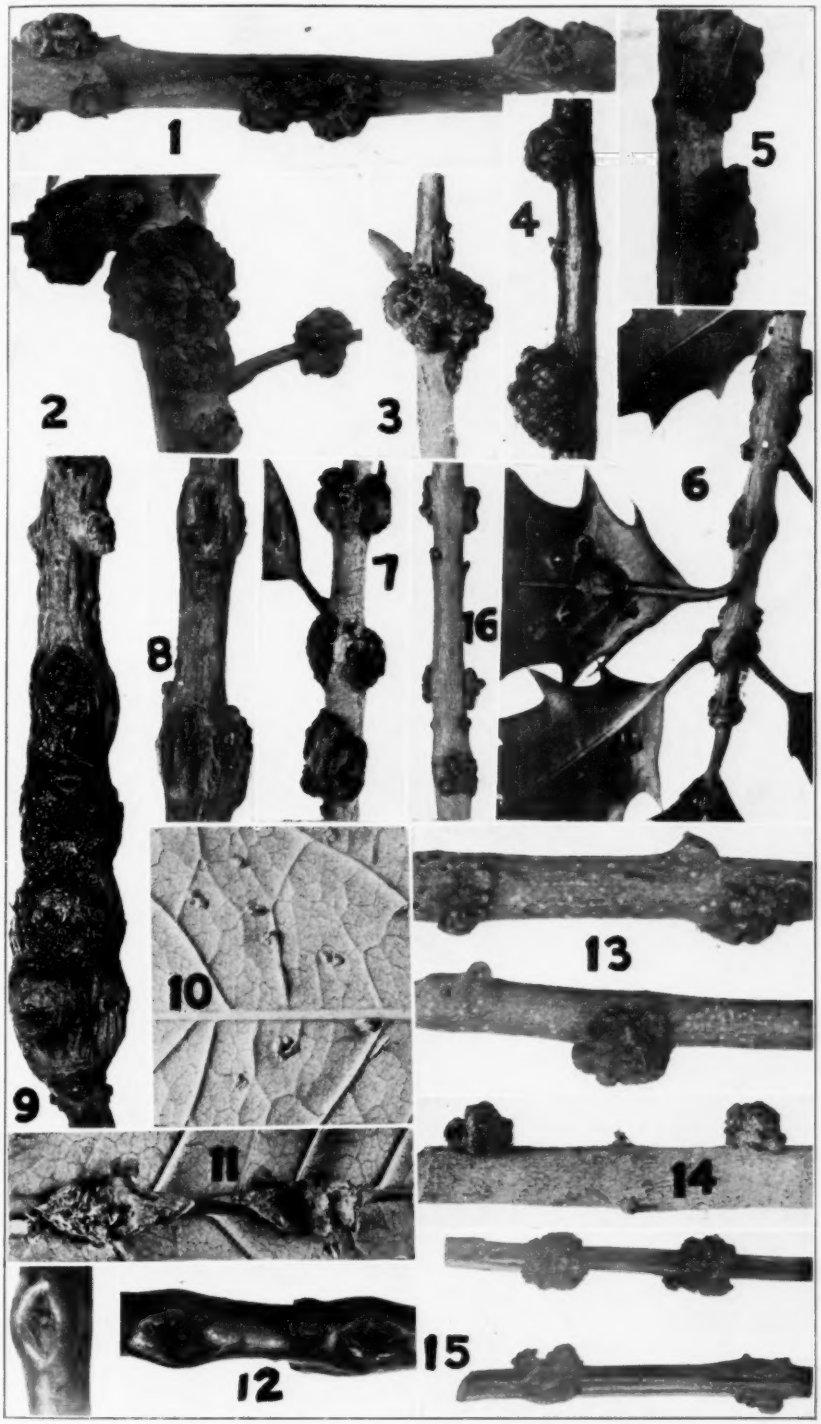
PLATE XXII. Cross Sections of Artificial Knots.

FIGS. 17–18. These two figures represent a single cross section, that was cut in two parts for arrangement on plate. They represent a cross section of knot at right on midvein, (Fig. 11) magnified about 15 diameters. The spaces have numerous bacteria in mass near the margins, especially the space shown in figures 17–18. The adjacent tissue is also invaded. The upper space of figure 18 has bacteria, but fewer in number. The black crosses in a general way indicate where the masses of bacteria are found. It is probable that both of the spaces were filled with the organisms which were largely washed away in staining and mounting.

FIG. 19. Cross section of knot of *Jasminum primulinium* similar to those of figure 15 magnified about 14 diameters. The crosses indicate approximately where the masses of bacteria are located. In the center is a mass of hypertrophied tissue partially surrounded by normal pith cells.

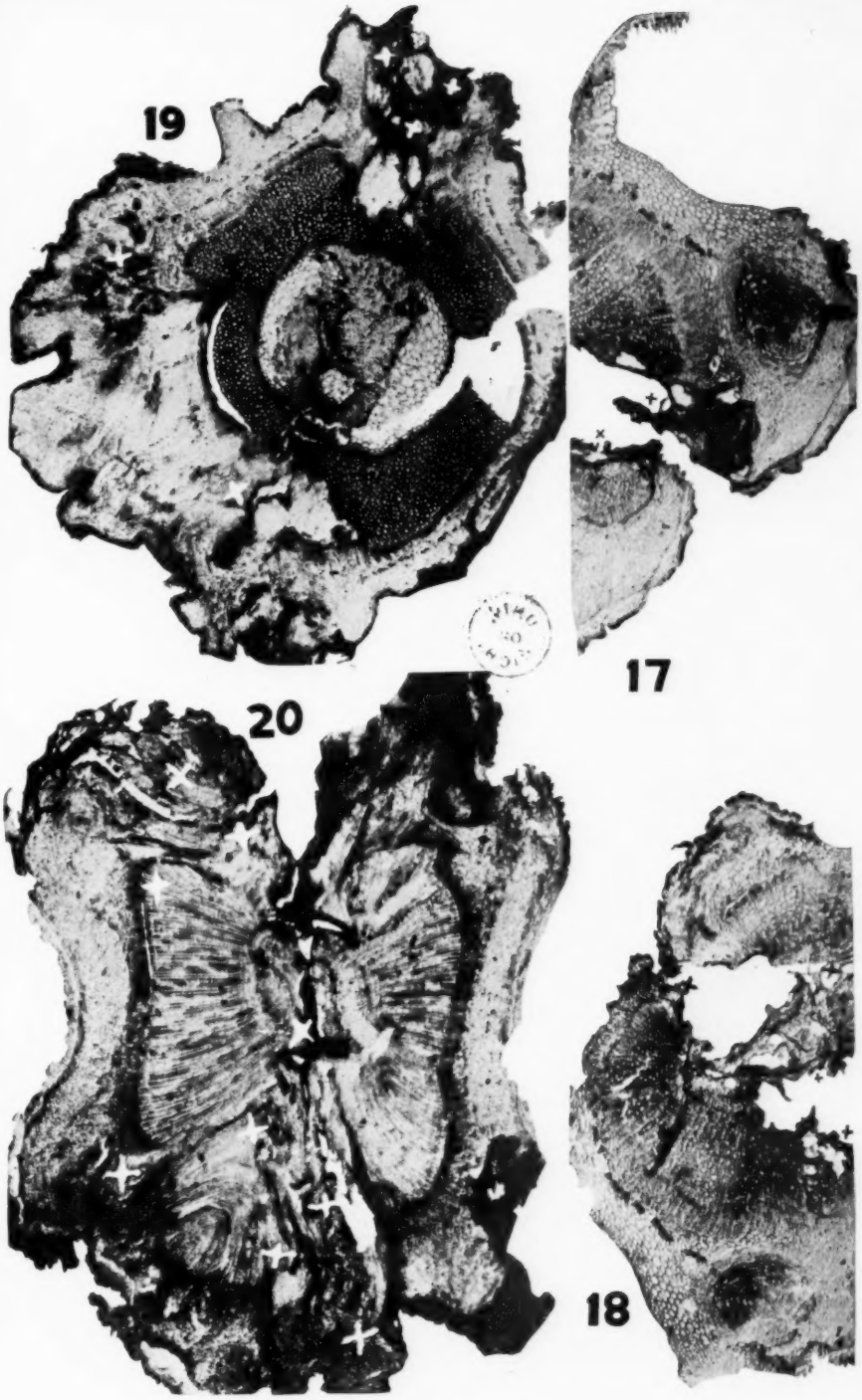
FIG. 20. A cross section of middle knot of figure 7 magnified about 12 diameters. The white crosses indicate the approximate location of the larger bacterial masses, which often take a deeper stain. The path of the inoculating needle is still evident.

[1] In the following legends the first date is that of the inoculation, the second the time when photographed.



OLIVE KNOT ON HOSTS RELATED TO THE OLIVE

UNIV. OF MICH.



CROSS SECTIONS OF HYPERTROPHIES.

UNIV. OF MICH.

ON THE USE OF THE ACETATES OF COPPER AS FUNGICIDES.

O. Butler and T. O. Smith

With One Figure in the Text

Two acetates of copper are used as fungicides, the normal or neutral acetate of copper Cu $(C_2H_3O_2)_2$ H_2O and the basic acetate of copper Cu $(C_2H_3O_2)$ CuO $6H_2O$ the former containing 31.8 per cent. metallic copper, the latter 34.4 per cent. Basic acetate of copper was introduced by Bencker[1] in 1889 and is still used especially by the grape growers of meridional France; neutral acetate of copper has been employed, according to Chuard and Dusserre[2], since 1892 in France at the Ecully School, and Briozi[3] has also used it with excellent results for the control of *Plasmopara viticola* in Italy. In the United States the copper acetates are practically unknown and we hardly find mention of them in the literature. Neutral acetate of copper was used by Galloway[4] in 1890 for the control of *Guignardia bidwellii*, and, at a strength of 1.13 per cent, gave the equivalent control of 3.4 per cent. Bordeaux mixture 1:0.66. Neutral acetate of copper has also been used as a spray for *Cylindro-sporium padi* on plum at a strength of 0.078 per cent and 0.156 per cent respectively[5] for *Entomosporium maculatum* on quince at strengths of 0.1 per cent, and 0.2 per cent.; for *Guignardia bidwellii Entomosporium* and scab on pear at 0.078 per cent.[6] In the case of the experiments with *Cylindrosporium padi* the acetate was compared with 0.078 per cent Bordeaux mixture 1:7.5 and certain other fungicides but in "none of the experiments could any very perceptible difference be noted between treated and untreated trees."[7] In the experiments with *Ento-*

[1] Bencker, Georges. Traitement du mildiou. Prog. Agric. et Vitic. **12:** (An. 6): 90–94. 1889.

[2] Chuard, E. and C. Dusserre. Sur les verdets employés dans la lutte contre le mildiou. Chron. Agr. Vaud **17:** 291–297. 1904.

[3] Briosi, Giovanni. Esperienze per combattere la Peronospora della vite. Att. 1st. Bot. Univ. Pavia (Ser. 2) **4:** 149–154. 1897.

[4] Galloway, B. T. Experiments in the treatment of plant diseases. Jour. Mycology **7:** 12–16. 1894.

[5] Galloway, B. T. Report on the experiments made in 1891 in the treatment of plant diseases. United States Dept. Agric. Div. Veg. Path. Bull. 3. 70 p. 1892.

From the method of preparation of the fungicide mentioned in the text (page 65) it is not likely that basic copper acetate was used.

[6] Loc. cit. supra, pages 65 and 10.

[7] Loc. cit. p. 63.

mosporium maculatum the acetates were compared with 0.103 per cent Bordeaux 1:1.33 the following results being obtained: The trees sprayed with the Bordeaux mixture produced 42.9 per cent more clean fruit than the non-sprayed trees; the 0.1 per cent neutral acetate produced 40.8 per cent. more clean fruit than the non-sprayed trees, and the 0.2 per cent wash 44.9 per cent. more clean fruit. In the experiments with *Entomosporium* and scab the 0.625 per cent. Bordeaux mixture 1:0.66 gave in the case of *Entomosporium* 21.2 times better protection than no spraying at all, whereas in the case of scab the amount of disease present was small and the copper acetate and Bordeaux mixture afforded a similar degree of protection. In the experiments on the control of *Guignardia bidwellii* 0.625 per cent Bordeaux mixture 1:0.66 was used but despite this fact the neutral copper acetate yielded a protection scarcely inferior to that obtained with the Bordeaux mixture.

Not only have the copper acetates been but little employed in the United States but formulae for their use are rarely seen in texts dealing with fungicides though curiously enough one generally finds a formula given for a cuprammonium. The cuprammoniums are used when a fungicide forming very inconspicuous spots on foliage or fruit is desired and yet the acetates of copper form even less conspicuous deposits, and, for the equivalent in copper, are many times less injurious to the plant sprayed than the former. In fact, and any one who so desires can readily verify the statement for himself, the acetates of copper are, when sprayed under conditions that permit drying of the foliage within an hour after the fungicide has been applied, no more toxic than a 1 per cent Bordeaux mixture, while it is unsafe to use cuprammonium sulphate (eau céleste), the least toxic of the cuprammoniums, at a greater strength than 0.25 per cent.[1] Furthermore, the acetates of copper are more adhesive than the cuprammoniums[2] and cost no more or but little more per equivalent of copper than cuprammonium sulphate which is the cheapest of the cuprammonium washes.

The evidence is abundant that the acetates of copper compare favorably with Bordeaux mixture in fungicidal properties. Foëx[3] tells us that the experimental trials made with basic copper acetate at the agricultural school of Montpelier have consistently given as satisfactory

[1] Butler, O. The cuprammonium washes. Phytopathology **7:** 235–268. Pl. 3–10. 1917. Bibliography, p. 267.

[2] Butler, O. and T. O. Smith. Relative adhesiveness of the copper fungicides. Phytopathology **9:** 431–444. 1919. Literature cited, p. 444.

[3] Foëx, Gustave. Cours complet de Viticulture. 4th ed. p. 579. Montpellier. 1895.

control as Bordeaux mixture and have even shown the former to possess greater adhesive properties. Viala[1] tells us that the numerous comparative experiments made during the last few years have shown clearly and unmistakably that basic acetate of copper washes constitute one of the most perfect methods of treatment of the downy mildew.

"For the last twelve years," Pacottet[2] emphatically remarks, "I have used it in Burgundy and have never seen the downy mildew. My grapes are the last to lose their leaves and some day I will show that it occupies the first rank in harmlessness to vegetation."

The acetates of copper, says Marsais,[3] are being increasingly used in viticulture.

Neutral acetate of copper was extensively used in the Canton de Vaud in 1904 and gave as satisfactory control as Bordeaux Burgundy mixture.[4]

Furthermore, the acetates of copper are non-toxic to the plant to be protected and form less conspicuous spots than the cuprammoniums. When we say that the acetates are non-toxic to the plant to be protected the reader should understand that we mean by this that they are non-toxic to the plant to be protected if the plant in question will stand spraying with Bordeaux mixture in which the ratio copper to lime is unity or less, but if Bordeaux mixture is injurious then the acetates will prove injurious also and may be found more injurious than 0.5 per cent or stronger Bordeaux mixture when used at equivalent strengths in copper. The acetates of copper are excellent fungicides. They compare favorably with Bordeaux mixture as regards efficiency and effectiveness and, while possessing all the fungicidal properties of the cuprammoniums, are not as toxic to the plant to be protected. For these reasons, therefore, we have thought it desirable to call attention to the copper acetates and particularly to the desirability of substituting them for the cuprammoniums.

Neutral copper acetate dissolves readily in cold water forming a clear bluish-green solution with faint acetic odor. When sprayed upon a plant the salt already in unstable equilibrium decomposes with the formation

[1] Viala, Pierre. Les maladies de la vigne. 3rd ed. p. 143 et seq. 1893.

[2] Pacottet, P. Sur le traitement du mildiou. Rev. Vitic. (An. 14) 27: 489–492. 1907.

[3] Marsais, P. Traitement du mildiou. Rev. Vitic. (An. 15) 29: 595. 1908.

[4] Chuard, E. and H. Faes. Enquete sur l'emploi du verdet neutre dans le vignoble vaudois en 1904. Chron. Agr. Vaud **17:** 601–608. 1904.

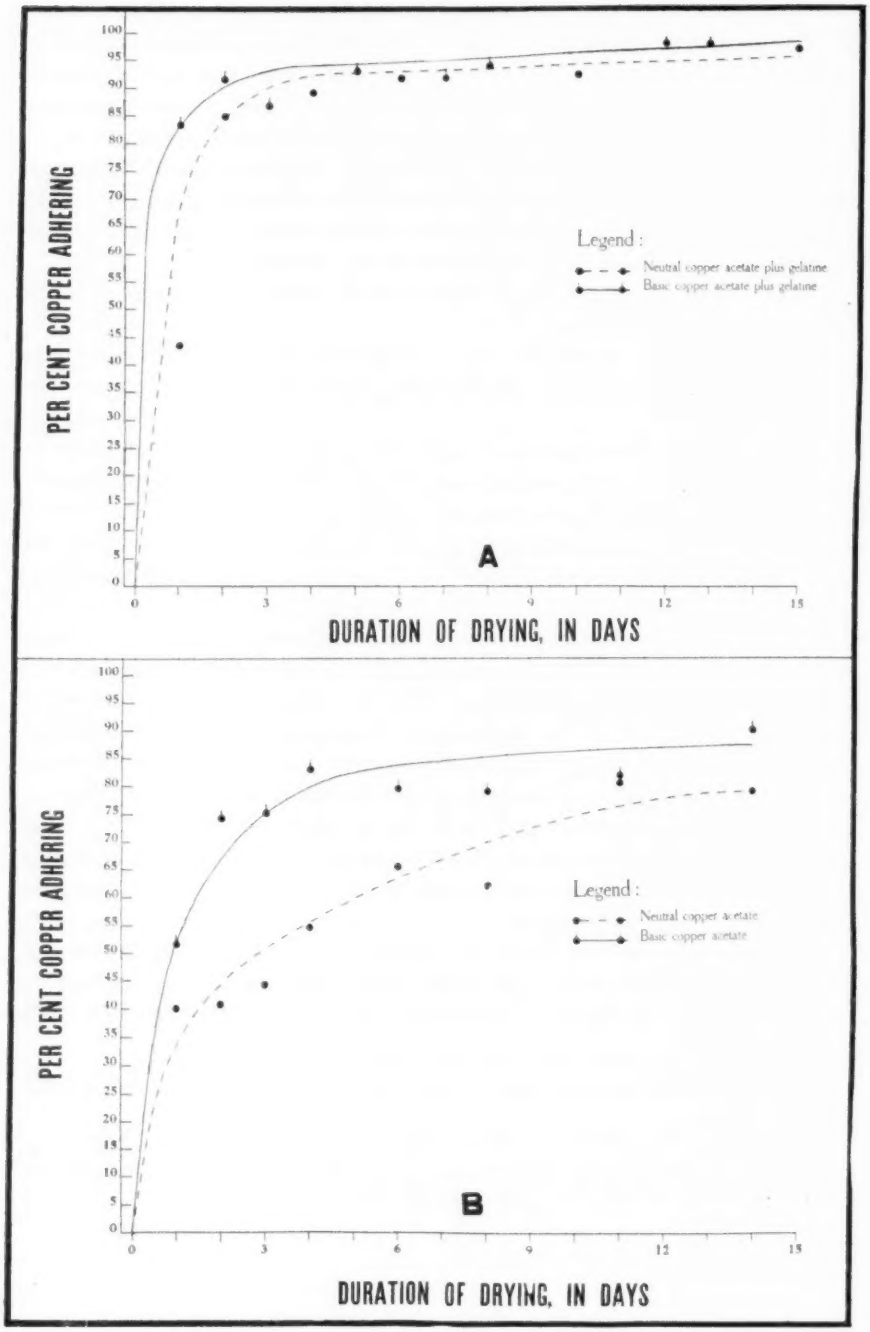


FIG. 1. Effect of length of time of drying with and without the addition of gelatine on the adhesiveness of the copper acetates.

as was first pointed out by Chuard and Porchet[1], of basic copper acetate mixed with hydroxides the degree to which the decomposition occurs depending on the temperature prevailing at the time the fungicide is applied. In cold weather the decomposition occurs more slowly and is less complete than in warm weather. Basic copper acetate dissolves more or less completely in cold water forming a blue-green solution with a faint acetic odor and decomposes more readily and completely on drying than does the neutral salt.

The adhesiveness of the acetates of copper depends on the degree to which they decompose on drying and on the length of time that elapses between time of application and time of first washing rain. The basic acetate of copper is more adhesive than the neutral acetate of copper and decomposes more rapidly on exposure to air than the latter as will be seen from the data presented in table 1.

The data given in the table was obtained in the following manner: Glass plates 5 inches square were thoroughly washed free from grease films, after which they were dried and set on a table adapted to support them at an angle of 20 degrees. The washes, made with chemically pure salts dissolved in distilled water, were sprayed on the plates by means of a hand sprayer and the spraying was continued until droplets approached the size at which they would coalesce and drain off the plates. After the plates had dried they were carefully examined for uniformity and divided into two lots of eight each. The plates in one lot were used for the determination of the total copper applied, while in the case of the plates of the other lot the copper adhering was determined after they had been exposed to artificial rain. The artificial rain used was ordinary tap water thrown from a Columbian lawn sprinkler standing on a level with the plates. The sprinkler occupied the center of a circle eight feet in diameter on the periphery of which the plates were placed facing inwards at a slight angle. The head of water used in running the sprinkler was regulated so that the water thrown fell by gravity upon the plates from a maximum height of 9 feet.

The data shows that the decomposition of the neutral acetate measured in terms of adhesiveness is roughly proportional to time while in the case of the basic acetate the rate of decomposition is extremely rapid during the first four days but continues from thence on more slowly. At no time does the adhesiveness of the neutral copper acetate equal

[1] Chuard, E. and F. Porchet. Recherches sur l'adherence comparée des solutions de verdet neutre et des bouillies cupriques, employées dans la lutte contre le mildiou. Compt. Rend. Acad. Sci. Paris **140**: 1354–1356. 1905.

TABLE 1

Effect of length of time of drying on the adhesiveness of 1 per cent neutral and 1 per cent basic copper acetates

A.—Neutral Copper Acetate

Interval between application of wash and incidence of rain	Duration of rainfalls	Amount of rainfall	Temperature of rain	Amount of copper sprayed on plates	Amount of copper adhering after rain	
Days	Minutes	Inches	° C.	Grams	Grams	Per cent
1	30	0.37	20° C.	0.0133	0.0053	39.85
2	32	0.43	24° C.	0.0123	0.0050	40.65
3	31	0.37	18° C.	0.0187	0.0083	44.38
4	32	0.43	22° C.	0.0141	0.0077	54.61
6	35	0.41	24° C.	0.0172	0.0113	65.69
8	30	0.38	23° C.	0.0167	0.0104	62.28
11	30	0.40	25° C.	0.0171	0.0138	80.70
14	34	0.32	18° C.	0.0187	0.0148	79.14

TABLE 1 (Continued)

B.—Basic Copper Acetate

Interval between application of wash and incidence of rain	Duration of rainfall	Amount of rainfall	Temperature of rain	Amount of copper sprayed on plates	Amount of copper adhering after rain	
Days	Minutes	Inches	° C.	Grams	Grams	Per cent
1	38	0.36	17° C.	0.0167	0.0086	51.49
2	30	0.45	23° C.	0.0194	0.0144	74.23
3	20	0.28	19° C.	0.0194	0.0146	75.26
4	31	0.48	20° C.	0.0155	0.0129	83.23
6	36	0.44	18° C.	0.0118	0.0094	79.66
8	58	0.79	18° C.	0.0135	0.0107	79.26
11	30	0.33	20° C.	0.0156	0.0128	82.05
14	39	0.55	20° C.	0.0269	0.0250	90.29

TABLE 2

Effect of length of time of drying on the adhesiveness of 1 per cent neutral and 1 per cent basic copper acetate containing .05 per cent gelatine

A. Neutral Copper Acetate

Interval between application of wash and incidence of rain	Duration of rainfall	Amount of rainfall	Temperature of rain	Amount of copper sprayed on plates	Amount of copper adhering after rain	
Days	Minutes	Inches	° C.	Grams	Grams	Per cent
1	30	0.46	19° C.	0.0177	0.0077	43.50
2	31	0.45	20° C.	0.0237	0.0201	84.81
3	32	0.49	18° C.	0.0187	0.0170	90.91
4	30	0.38	19° C.	0.0223	0.0199	89.24
6	30	0.41	21° C.	0.0228	0.0209	91.67
8	30	0.32	19° C.	0.0225	0.0211	93.78
10	32	0.46	19° C.	0.0263	0.0243	92.39
15	30	0.45	23° C.	0.0177	0.0172	97.12

B. Basic Copper Acetate

1	42	0.43	17° C.	0.0156	0.0136	83.33
2	35	0.32	17° C.	0.0144	0.0129	89.58
3	30	0.40	21° C.	0.0228	0.0198	86.84
5	30	0.43	22° C.	0.0233	0.0217	93.13
7	31	0.53	22° C.	0.0162	0.0149	91.97
8	31	0.40	21° C.	0.0225	0.0210	94.27
12	30	0.46	22° C.	0.0251	0.0248	98.01
13	31	0.41	22° C.	0.0246	0.0241	97.97

that of the basic acetate of copper. The facts brought out in the table are more strikingly shown in figure 1, B. Adhesiveness is an important requisite in a fungicide and should only be sacrificed when this sacrifice is made in order to secure an important property that would not otherwise be had. In the case of the acetates of copper a sacrifice of adhesiveness is only justified if increased toxicity of the unit copper is thereby secured. In other words, in order for the neutral acetate of copper to be of commensurate value to the basic acetate of copper in practise it should be able to prevent the germination of fungous spores not affected by the latter. Obviously the spores in question will have to be unaffected by small amounts of soluble copper. The uredospores of *Puccinia antirrhini*, for example, fulfill the conditions required. When neutral and basic acetate of copper are tested following the method of Reddick and Wallace[1] and using the uredospores of *P. antirrhini* we have not found the neutral acetate to possess measurably greater toxicity than the basic acetate. We would be inclined, therefore, to rule neutral acetate of copper off the list of fungicides were it not for the fact that it is more readily obtained than the basic acetate and can have its adhesiveness greatly increased at small expense by the addition of 0.05 per cent. of gelatine. The use of gelatine for the purpose of increasing the adhesiveness of acid washes was first proposed by Vermorel and Dantony[2] and when used with the acetates of copper proves extremely beneficial. We have shown in a previous paper[3] that gelatine increased the adhesiveness of neutral copper acetate 14.4 per cent. and the basic acetate 12.3 per cent when the fungicides were applied to Coleus leaves. In figure 1, A we give the results obtained on glass plates. The data shows very clearly that the addition of 0.05 per cent. gelatine very materially increases the adhesiveness of the salts, the adhesiveness of neutral copper acetate at the end of forty-eight hours being virtually the same as that of the basic acetate and only slightly lower at each of the subsequent periods of observation. The adhesiveness of neutral copper acetate plus gelatine after standing twenty-four hours is 9.1 per cent. greater than that of the wash prepared without gelatine, but after forty-eight hours the difference in favor of the wash plus gelatine reaches the imposing figure of 108.6 per cent. In the case of the basic acetate of copper the increase in adhesiveness brought about by the addition

[1] Reddick, Donald and Errett Wallace. On a laboratory method of determining the fungicidal value of a spray mixture or solution. Science 31: 798. 1910.

[2] Vermorel, Victor and E. Dantony. La défense de nos jardins contre les insectes et les parasites p. 199. Villefranche, Bur. Progres Agr. and Vit. 1914.

[3] Loc. cit. ante. p. 441.

of gelatine is 61.8 per cent. at the end of the first day of drying and only 20.6 per cent. at the end of the second. The decomposition of the basic acetate occurs mostly during the first twenty-four hours following the application of the wash while in the neutral acetate the decomposition is greater during the second twenty-four hours. It is, of course, desirable to have a fungicide reach its maximum adhesiveness as soon after drying as possible, hence even with the addition of gelatine the neutral acetate of copper is slow in reaching the desired state and in this respect is noticeably inferior to the basic acetate. Neutral acetate of copper plus gelatine is not as adhesive as basic acetate of copper plus gelatine, the inferiority being especially marked for periods following the application of the wash of less than forty-eight hours, but as will be seen from a consideration of figure 1, is so superior to the basic acetate without the addition of gelatine that it deserves to be introduced into our formulae as a substitute for use when the latter is not obtainable.

It follows from the study we have just made of the acetates of copper that these salts are deserving of a position in our fungicide formularies. We propose, therefore, the following formulae. The weaker strength is intended for use when the acetate is to be used in lieu of a cuprammonium, the stronger when it is desirable to use a colorless wash in lieu of Bordeaux mixture and of the same fungicidal value.

Stock solutions of the copper acetates should be prepared by suspending the salts in a gunny sac or cheese cloth bag near the surface of the water following the procedure in common use when making a stock solution of copper sulphate. The water used should be cold (15° C.). No attempt should be made to hasten dissolution by means of hot water as the salts will decompose and decomposition greater than that due to the hardness of the water is to be avoided. The stock solution should be made to contain 1 pound per gallon. There will, then, be required for 50 gallons of wash.

Water	49 gallons	46 gallons
Basic acetate copper (stock solution)	1 "	4 "

When gelatine is added to increase adhesiveness always required when the neutral acetate is used, the formula becomes

Water		48 gallons	45 gallons
Stock solution	basic acetate	1 "	4 "
	neutral acetate		
Stock solution of gelatine		1 "	1 "

The stock solution of gelatine is made by dissolving 4 ounces of leaf gelatine in 5 quarts boiling water, cooling to about 40° C. then adding

4 quarts of this stock solution to the acetate previously diluted with the water and thoroughly stirring. This is most readily accomplished if the solution of gelatine is added after the water and the stock solution of acetate have been placed in the spray tank.

SUMMARY

1. The acetates of copper are excellent fungicides and deserve especial consideration when a colorless deposit is required.

2. The acetates of copper are less injurious to the plant sprayed than the cuprammoniums and are to be preferred to them.

3. Basic acetate of copper adheres much better than the neutral acetate of copper.

4. An addition of gelatine increases the adhesiveness of the acetates markedly, the greatest benefit occurring to the neutral acetate.

NEW HAMPSHIRE AGRICULTURAL EXPERIMENT STATION
DURHAM, N. H.

THE GROWTH OF THE WHEAT SCAB ORGANISM IN RELATION TO HYDROGEN-ION CONCENTRATION.

JEAN MACINNES

WITH ONE FIGURE IN THE TEXT

A strain of Fusarium, isolated from scabby wheat in Minnesota, has been found to be capable of growing in solutions varying through an

TABLE 1

Character of growth of the wheat scab organism in relation to hydrogen ion concentration

	Buffer solution (c. c.)		Sodium hydroxide (c.c.—1N)		Distilled water (c. c.)		Czapek's solution (c. c.)		Initial pH[2]		Character of growth[3]	
Expt. No. Flask No.	1	2	1	2	1	2	1	2	1	2	1	2
1	1	1	0	0	35	45	10	10	3.0	3.0	very slow	very slow
2	1	1	5	5	30	40	10	10	3.5	3.9	slow	slow
3	1	1	10	10	25	35	10	10	4.6	4.9	good	good
4	1	1	15	15	20	30	10	10	5.8	6.	good	good
5	1	1	20	20	15	25	10	10	7.2	7.1	good	good
6	1	1	25	25	10	20	10	10	9.0	9.8	good	good
7	1	1	30	30	5	15	10	10	10.6	?	slow	slow
8	1	1	35	35	0	10	10	10	12.0[1]	11.7	very slow	very slow
9	–	1	–	40	–	5	–	10	–	11.9	–	none
10	–	1	–	45	–	0	–	10	–	12.1	–	none

[1] Approximate.

[2] Average of at least 2 determinations.

[3] Experiment 1 run in triplicate, Experiment 2 with 6 similar flasks for each set.

unusually wide range of hydrogen-ion concentration. The organism grew in nutrient media ranging from pH 3.0 to pH 11.7 (Tab. 1). Definite results have not yet been obtained for concentrations immediately below pH 3.0 or above 11.7, but indications are that these figures are close to the acid and alkaline limit for growth.

The organism was grown in a modified Czapek's solution[1] to which a mixed buffer solution was added, the pH being varied by the addition of increasing amounts of sodium hydroxide.[2] The buffer solution was made up as follows:

5.252 gm. citric acid
1.876 gm. glycocoll
2.269 gm. KH_2PO_4
50 cc. distilled water

The various constituents of the medium were sterilized before mixing in order to prevent the breaking down of the sugar which takes place in the more alkaline solutions when heated.

The solutions, in 56 c. c. portions, were mixed in sterile flasks of 150 c. c. capacity and left to incubate for at least 24 hours in order to insure sterility. Flasks showing cloudiness or other contaminations were discarded. The inoculations were made with young rapidly growing mycelium of the organism taken from potato dextrose slants. Uninoculated flasks, in general, remained sterile throughout the two or three weeks during which the experiments were conducted.

The hydrogen-ion concentration of the solutions was determined at the beginning of the experiments and at various intervals thereafter by means of a Clark (4) hydrogen electrode, including the shaking cell as described by him. As growth proceeds, the hydrogen-ion concentration of the solutions is changed by the organism, those on the acid side tending to become more alkaline and those on the alkaline side more acid. Further work on the change of pH by the organism is in progress.

[1] Modified Czapek's solution as used:

$MgSO_4$	1.0 gm.
KC	1.0 gm.
$FeSO_4$	0.02gm.
$NaNO_3$	4.0 gm.
Sugar	30.0 gm.
Distilled water	1 liter.

Sterilized at 5 lbs. pressure for one half hour.
(The phosphate was omitted due to its presence in the buffer solution).

[2] The sodium hydroxide stock solution was prepared in the manner suggested by Clark (4), in order to prevent the formation of carbonates by the exposure to the CO_2 of the air.

It is interesting to compare the pH range of this organism with that of other micro-organisms. Hopkins (10), working with a wheat scab organism (*Gibberella saubinetii*), obtained growth in nutrient solutions varying in hydrogen-ion concentration from pH 2.5 to pH 5.5, the upper limit not being determined. Brightman, Meachem, and Acree (2) found that the organism which causes chestnut blight (*Endothia parasitica*) grew in solutions varying in hydrogen-ion concentration from pH 4.0 to pH 8.5. Wakeman (18) in determining the changes in

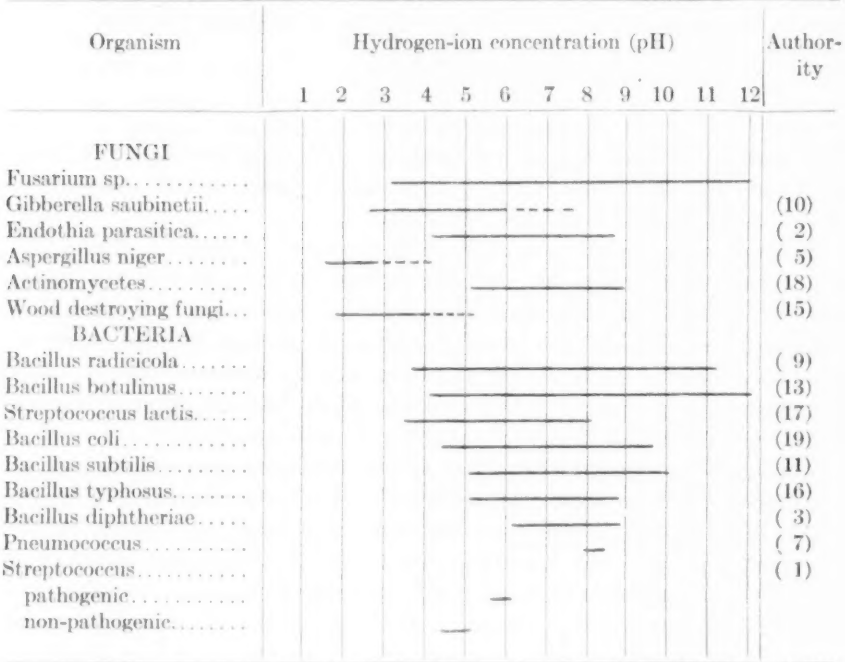


FIG. 1. The Relation of a Number of Pathogenic Micro-organisms to Hydrogen-ion Concentration.

reaction due to the growth of Actinomycetes, found that the acid and alkaline limits for growth were between pH 5.0 and pH 8.7, varying somewhat with the species. Meacham (15) found that four common wood destroying fungi develop normally in media having a very high acidity (pH 1.7) but no data are given for the alkaline limit. *Aspergillus niger* is reported by Currie (5) to grow at pH 1.4–1.6, the critical alkaline concentration not being determined.

Much more work has been done with bacteria in connection with

tolerance to hydrogen-ion concentration, especially those bacteria causing human and animal diseases. An excellent summary of this work is given in a recent article by Dernby (6), in which he shows that none of these organisms will grow in a medium more acid than pH 4.5 or more alkaline than pH 9.0. Fred and Loomis (9), on the other hand, found that *Bacillus radicicola* developed normally at all hydrogen-ion concentrations between pH 3.5 and pH 11.1. The only other reference to organisms tolerating as wide a range of hydrogen-ion concentration as that of the Fusarium reported in this paper is that of Itano, Neill, and Garvey (13). Some strains of *Bacillus botulinus* were found by them to grow in acid solutions of pH 4.0, while others would not grow in solutions more acid than pH 6.0. On the alkaline side, some strains would develop normally at pH 12.0 but others not above pH 9.0. According to Wyeth (19) *Bacillus coli* can grow at pH 4.27 to pH 9.87, and Itano (11) has given the range for *Bacillus subtilis* as being from pH 5.0 to pH 10.0. On the other hand, the more highly specialized Streptococci studied by Ayers, Johnson, and Davis (1) have been found extremely sensitive to slight changes in hydrogen-ion concentration and to develop normally only between pH 5.4 and 6.0.

A graphic representation of the acid and alkaline limits of a number of common bacteria and of all the fungi mentioned above is given in figure 1. It is evident that the organism used in these experiments is exceptional, particularly among the fungi, in the wide range of hydrogen-ion concentration in which it will grow. This organism is known to be capable of attacking a very large number of hosts.[1] If other more specialized Fusaria are shown to be more limited in their relations to hydrogen-ion concentration these results would seem to be of special significance.

Department of Biology
Massachusetts Institute of Technology
Cambridge, Mass.

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SOME EXPERIMENTS WITH AZUKI-BEAN MOSAIC

TAKASHI MATSUMOTO

In a field at Morioka College of Agriculture and Dendrology a considerable percentage of a typical mosaic disease of Azuki beans, *Phaseolus radiatus*, var. *aurea*, was found in July, 1921. The disease spread very rapidly, especially in a field of the variety Kensaki so that about 70 per cent of the plants were more or less affected before harvest time. The disease is a typical mosaic and resembles that of soybean mosaic as described by Gardner and Kendrick[1]. The diseased plants are more or less stunted, and sometimes are accompanied by a slight distortion of young leaves. The leaflets present an unmistakable mottling which is usually more pronounced on young leaves. The dark green parts of the affected leaves are slightly raised above the surrounding surface, thus giving the so called "puffy" appearance. In most cases the dark green parts predominate, and sometimes present map-like features, being intermingled with light green parts.

Anatomical structures of the diseased leaves. Materials were fixed in absolute alcohol containing 25 per cent of glacial acetic acid, afterwards they were cut with a microtome and the sections were stained with Delafield's haematoxylin. The anatomy of the diseased leaves is found to be identical with that of cucumber mosaic as described by Doolittle[2]. The mesophyll of the dark-green parts of the diseased leaves is strikingly thicker than that of the light-green or yellowed parts (Figs. 1, 2). The palisade cells of the former are markedly longer and narrower than those of the normal tissues. The corresponding cells of the light-green parts are much shorter than those of the normal ones, more or less isodiametric, and show a tendency to crowd together so closely that their intercellular spaces are almost invisible. The second important difference between these two different portions occurs in the spongy parenchyma. The spongy parenchyma of the light-green parts is also strikingly compact as compared with that of the dark-green areas. The chloroplasts of the former, as in cucumber mosaic, are slightly smaller and less in number than in the darker areas.

Starch and sugar in mosaic area. Starch tests were made after the

[1] Gardner, Max W. and James B. Kendrick. Soybean mosaic. Jour. Agric. Res. **22**: 111–113. 1921.

[2] Doolittle, S. P. The mosaic disease of cucurbits. U. S. Dept. Agric. Bull. 879. *69 p.* 1920.

method described by Freiberg[1]. Sections of the diseased leaves were mounted in water, and the slide drawn through the flame of a microburner until the drop of water began to simmer. A drop of 75 per cent.

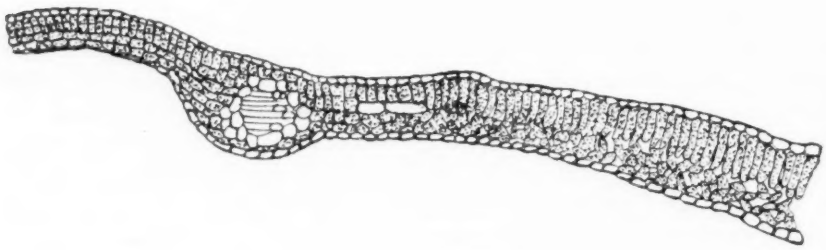

FIG. 1. THE MESOPHYLL OF MOSAIC AZUKI BEANS. RIGHT, DARK-GREEN PORTION; LEFT, LIGHT-GREEN OR YELLOWED PORTION

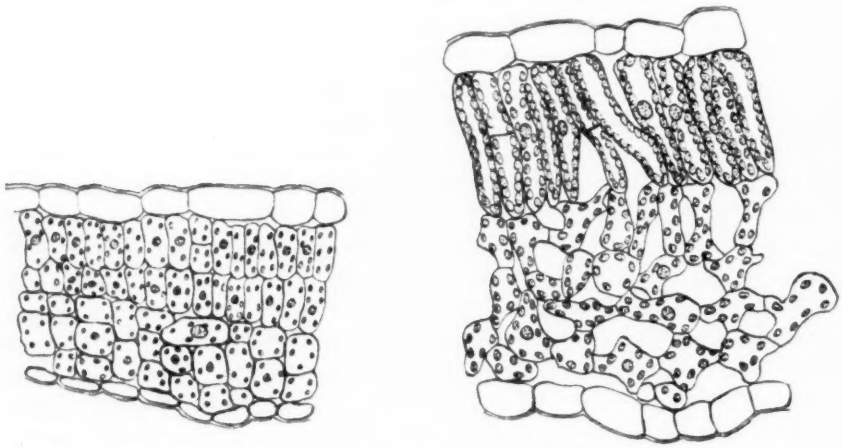

FIG. 2. THE MESOPHYLL OF MOSAIC AZUKI BEANS. RIGHT, DARK-GREEN PORTION; LEFT, LIGHT-GREEN OR YELLOWED PORTION.

alcohol and a drop of standard iodine were then added, after which the sections were examined under a microscope. In this simple test it was readily determined that starch in the dark portions is more abundant than in the light-green parts. Woods[2] found that the light-green parts

[1] Freiberg, Geo. W. Studies in the mosaic disease of plants. Ann. Missouri Bot. Garden **4**: 175–232. 1917.

[2] Woods, Albert F. U. S. Dept. Agric., Bur. Pl. Ind. Bull. 18. *24 p.* 1902.

of the mosaic leaves of tobacco picked early in the morning had a higher starch content than the dark-green areas, as indicated by iodine tests, and attributed this fact to an inhibitory action exerted on diastase by oxidase. The observations of the writer, however, accord with those of Freiberg and lead to the conclusion that starch is more abundant in the dark parts, regardless of the time of day. This evidence is more clearly and decidedly assured by comparing a mosaic leaf treated with iodine solution with a picture of the same leaf which has been photographed just before a treatment with alcohol for extracting chlorophyll.

Attempts were made to determine quantitatively the amount of sugar in the two portions by using the well-known Fehling's solution. For this particular study this method was not very satisfactory, owing to the great diffusibility of the sugar in the tissues of the leaves. Nevertheless from careful repetitions it is concluded that sugar is more abundant in the dark-green parts than in the light-green parts.

Varietal susceptibility. No extensive tests on varietal susceptibility have been made but some attempts have been made in a limited area of a test garden. In this connection thanks are due to Mr. Kenkichi Sato, a colleague.

The following 23 varieties were under observation. Of 25 plants of each variety examined the percentage of mosaic was found to vary from no infection at all to 100 per cent. infection. The percentage infection in each variety is indicated by the numeral following the name of the variety. Aka-azuki, 4; Benizu, 0; Bon-Azuki, 24; Dainagon, 8; Higashinimen, 12; Chin-yen, 0; Kensaki, more or less in all plants; Koshiroazuki, 16; Kwa-gin, 16; Maruba, 4; Min-zan, 0; Vuminza, 4; Mumeimadara, 4; Muroran, 4; Okute-azuki, 8; Ri-don-yo, 0; Ri-kwan-don, 0; Rin-san-in, 0; Ryokuzu, 0; Shiboriwake, 8; Shiro-azuki, 8; Shirosaya, 4; Wasedainagon, 0;

As will be seen, some varieties were free from the disease. It remains to be determined, however, whether the varieties are entirely immune or not. Attempts to communicate the disease to related species and to cucurbits are in progress.

MORIOKA, JAPAN.

POTENTIAL SPORIDIA PRODUCTION PER UNIT IN CRONARTIUM RIBICOLA

MINNIE W. TAYLOR

WITH ONE FIGURE IN THE TEXT

Actual counts of telial columns were made on 68 leaves of 12 species of Ribes—*R. americanum*, *R. cynosbati*, *R. glandulosum*, *R. lacustre*, *R. nigrum*, *R. odoratum*, *R. oxyacanthoides*, *R. rotundifolium*, *R. setosum*, *R. triste* and *R. vulgare*—taken from the field collections in the Office of Forest Pathology of the United States Department of Agriculture at Washington, D. C. These leaves were selected on the basis of the average size and the maximum infection represented in the collections. The writer is indebted to Dr. Perley Spaulding and to Dr. R. H. Colley for valuable advice and assistance.

The average number of teliospores in a single telial column was estimated by counting the spores in a series of cross and longitudinal sections of telial columns from *R. nigrum*. Each spore was considered as having a potential capacity for producing 4 sporidia. The number of spores was estimated to be 1500 with 6000 potential sporidia per column. These constants were used in estimating the spores and potential sporidia in the columns from all the species studied with the exception of those from *R. glandulosum*. The columns of this latter species were conspicuously longer and narrower than those of the *R. nigrum* type. Therefore, separate counts were made and the estimates of 936 spores with 3744 potential sporidia per column were obtained.

The area of all leaves of each species was determined in square inches by planimeter measurement. Actual count was made of every telial column on every leaf of each species examined and the number of teliospores estimated. The average number of teliospores per square inch per species was obtained by dividing the total count per species by the total leaf area per species. The quotient multiplied by 4 gave the average number of potential sporidia per square inch of leaf surface. This last figure was used as the unit for comparison.

The average number of potential sporidia per square inch of leaf surface for each of the 12 species of Ribes studied is shown below:

Ribes lacustre	281,280
" *triste*	360,000
" *setosum*	756,540
" *americanum*	974,280
" *cynosbati*	1,141,920

Ribes vulgare	1,305,000
" *glandulosum*	1,679,484
" *rotundifolium*	2,746,140
" *oxyacanthoides*	3,706,020
" *odoratum*	5,480,640
" *aureum*	5,645,520
" *nigrum*	16,799,400

As previously stated, the above figures are based on actual counts of telial columns on representative Ribes leaves in the herbarium collections. Since these collections were not made with this study in view and do not always show average field conditions, it is obvious that the figures cannot be applied rigidly in estimating the relative potential danger of any given species of Ribes.

Field notes indicate that *R. americanum* is seldom found infected unless it happens to be in the immediate vicinity of diseased pines, in which case the infection may be quite heavy. Moreover, under local conditions, *R. vulgare* may occasionally be found heavily infected. In the middlewest, *R. cynosbati* seems to be more susceptible than *R. glandulosum*.

In order to arrive at definite constant figures, it would be necessary to make a study of material collected for this particular purpose from average bushes of each species, taking into account the various localities in which the species occur, the average size of bush for each species, the average size of leaf for each species, the average susceptibility of the species and the average amount of infection per bush per species. This would be a difficult task and until such an ideal collection of material is available, the information obtained from these herbarium collections can be used as a basis for a general estimate of the potential sporidia production for the species studied.

A subsequent study, based upon material actually collected for the purpose, will doubtless show certain changes in the relative positions of the species but the indications are that the cultivated *R. nigrum* will still head the list in potential sporidia production per square inch of leaf surface and when present must be regarded as the most dangerous Ribes neighbor of the white pine.

OFFICE OF FOREST PATHOLOGY
BUREAU OF PLANT INDUSTRY
UNITED STATES DEPARTMENT OF AGRICULTURE
WASHINGTON, D. C.

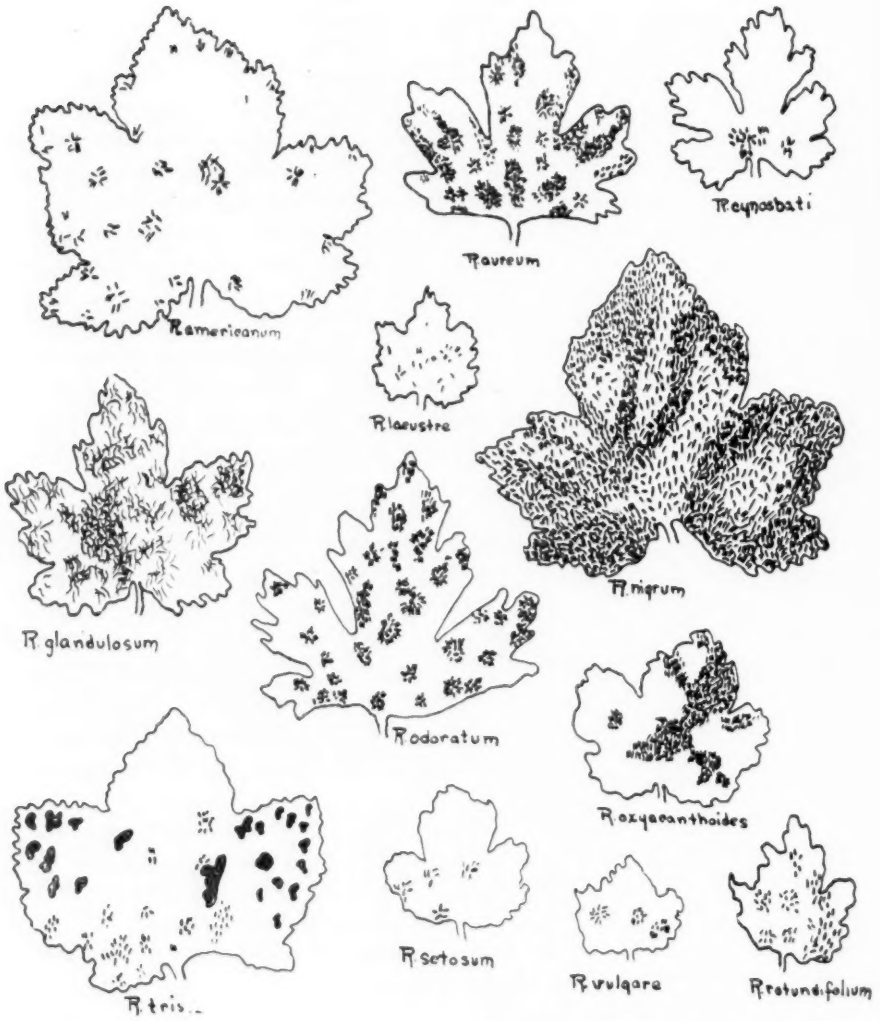

Fig. 1–Diagramatic drawings to show average shape and size and relative amount of infection of the twelve species of Ribes leaves studied. ½ natural size.

Note–The leaves of *R. triste* contained many holes—due probably to a "shot hole" fungus—which were not taken into consideration in computing the area.

PHYTOPATHOLOGICAL NOTES

Relation of plant pathology to the newly organized Science Service. No argument is needed to convince plant pathologists of the desirability of greater publicity for results of research. It is fully evident that a more complete knowledge of plant pathology and its accomplishments on the part of the general public will be helpful not only to the public itself, but also to the science through increased encouragement and support for our projects. Various individuals, both scientific and non-technical, have written popular articles on subjects pertaining to plant pathology. The merit of these articles has varied widely, and the total number appearing in the newspapers or in publications outside of experiment station literature, is small. Most plant pathologists are engrossed in their work and few have had experience in writing for the popular reader. Hence, this type of composition has been neglected and plant pathology has suffered through lack of understanding and appreciation on the part of the public.

In the recently organized Science Service, a new channel has been opened for bringing the results of scientific investigation before the public in a quick, accurate, and comprehensible way. This service, which has recently been described in Science,[1] has been made possible through the generosity of Mr. E. W. Scripps of California and is organized with headquarters in the National Research Council building at Washington. It is governed by a board of fifteen trustees of whom ten are scientists, and five are journalists. Dr. Edwin E. Slosson, who for twelve years was Professor of Chemistry in the University of Wyoming and for seventeen years was editor of *The Independent* of New York, has been selected editor. The corporation is non-profit making, all receipts from sale of articles, books, films, etc. being used to develop the service.

The object of Science Service may be given in the words of Doctor Slosson:[1]

"Science Service has been founded for the purpose of reaching through the press and motion pictures a wider public than is now interested in the progress of science. The only way to eliminate fake science stories that too often appear in our papers is to crowd them out with articles that are more accurate and equally interesting. . . . Science Service will spare no pains or expense in the effort (1) to give the best possible quality of reading, and (2) to give it to the largest possible number of readers."

[1] Slosson, Edwin E. A new agency for the popularization of science. Science n. s. **53**: 321–322. Feb. 8, 1921.

At present Science Service is selling to a large number of newspapers a weekly news bulletin, the items in which range in length from a single sentence to 150 or 300 words. Writers of these articles are paid one cent a word. Longer articles of from 500 to 1500 words are bought by Science Service at two cents a word and used in newspapers and magazines.

This outlet for popular articles on plant pathology presents an excellent opportunity to us to get our information before the people in an easy, quick, and effective way, and we should by all means take full advantage of it. Plant Pathology, as always, should be at the front in movements of this kind. Persons interested should write immediately to Doctor Slosson and ask him for further directions as to what kind of articles he wants and how they should be written. If it is not possible for the contributor himself to write his article in finished form for publication, data should be submitted to be worked up by the editorial staff of Science Service. Lack of time or skill in writing popular articles need deter no one from doing his share in properly presenting plant pathology to the general public.

It is preferred that contributors send their papers directly to the Editor of Science Service, 1701 Massachusetts Ave., Washington, D. C., but the writer who is interested in the project as a part of the program of the Advisory Board will be glad to act as an intermediary between the members of the American Phytopathological Society and Science Service in so far as may be desired.

R. J. HASKELL, Secretary,
Advisory Board of American Plant Pathologists.

The occurrence of Cornartium ribicola in Europe.—The aecidial stage of *C. ribicola* chiefly occurs in Europe on the Weymouth pine (*P. strobus*), although *P. lambertiana* and *P. flexilis* may also be infected, as reported lately by Moir[1]. The rust is recorded by Grove as distributed over Europe, north of the Alps, it is not listed by Fragoso among fungi of the Penninsula, and we never observed it in southern or central France. In French forests, Weymouth pines occur chiefly in the Vosges; they were planted extensively in Sphagnum moors covering 800 hectares around Epinal. Cases of infection by *C. ribicola*[2] are recorded by Forester M. Mangin, chiefly on the border of the forests.

[1] Moir, W. Stuart. [Sur l' extension du *Cronartium ribicola* Diete]. (Abstract of letter.) *In* Bul. Soc. Path. Veg. France, t. 7, fasc. 4, p. 100. 1920.

[2] Hariot, P. Sur quelques rouilles des plantes cultivées. (Abstract.) *In* Bul. Soc. Path. Veg. France, t. 1, fasc. 1, p. 15–16. French abstract *in* Min. Agr. [France], Ann. Serv. Epiphyties, t. 3, 1914. p. 22–23. 1916.

Well grown trees, averaging 0.20 m. in diameter at base may be infected to the top, as well as seedlings in nurseries. Once initiated, infection spreads rapidly; visiting a place where one of the pines was rusted in a private forest, Forester Mangin truly prophesied that all of them would die within two years. In governmental forests, however, Mangin does not consider the rust a menace, or even a serious trouble, as simple sanitary measures always restrict the infection to a few single trees; infected pines are felled and burned as soon as located, while the brushwood in the vicinity is destroyed, by assartage, to get rid of the telial hosts. A specimen of infected trunk was deposited by Mangin at the Laboratoire de Cryptogamie du Museum d'Histoire Naturelle de Paris, Specimens of *Peridermium strobi* were forwarded to the Station de Pathologie Végetalé but rarely, as the disease is causing but little trouble in France; aeciospores from a Peridermium obtained in the Vosges, though having been travelling for a week, developed uredo sori of Cronartium on *Ribes uva-crispa*, within ten days of inoculation. *Peridermium strobi* was also reported from the forest of St. Amarin, Alsace.

The telial stage is not common; we never found it on wild Ribes, but it rusted cultivated Ribes, and more severely, *R. nigrum* of the Anjou, in 1917[1].

We are indebted to Fischer[2] for important data about the European distribution of *Cronartium ribicola*. He first reported the rust on *Ribes petracum* in the Engadine (1895), where Schellenberg found the aecial stage in 1903 on *Pinus cembra*. From the Engadine, where Fischer considers the rust indigenous, it never spread to the rest of Switzerland. However, infected seedlings of *P. strobus* imported from Germany, started a rust epidemic from the Locle, in the year 1904–05; this epidemic spread rapidly all over Switzerland. Rust damage was complained of in Boudry (Neufchatel, 1909), in Montagny, and in Bern (1911), and even to Chateau d'Oex (1915). In the district of Zurich, where the disease is most severe, the Weymouth pine is planted no more. Curiously enough, this epidemic was demonstrated by Fischer actually to be of foreign origin, having originated in Siberia, and spread to Switzerland through Germany, whereas *Cronartium ribicola* from the Engadine remained restricted to that district.

Ribes nigrum and *R. aureum* are most severely rusted, and *R. sanguineum*

[1] Marchal, Paul, and Arnaud, G. Rapport phytopathologique pour les années 1916 et 1917. Groseilliers. *In* Min. Agr. [France], Ann. Serv. Epiphyties, t. 5, 1916–17, p. 29. 1918.

[2] Fischer, Ed. Pilze. *In* Ber. Schweiz. Bot. Ges., Heft 24–25, p. 72. 1915–16.

somewhat less so. Weymouth pines are chiefly rusted when 15 to 50 years old; completely girdled shoots are sure to die quickly; partially infected shoots may linger for months, or perhaps recover, if diseased parts be carefully excised. Good results were reported from the Belgian Arboretum of Groenendael, due to the following treatment: the rusted cortex was removed, and the wound was washed with a 10 per cent solution of potassium permanganate. To restrict the spread of *C. ribiculom*, Badoux[1] writes as follows:

In nursery, use only healthy, clean, and superficially disinfected seeds.

Plant healthy seedlings, obtained from uninfected nurseries.

Do not grow Ribes in the vicinity of Weymouth pine nurseries.

Do not grow Weymouth pine in infected nurseries, for some years at least.

Plant Weymouth pines among other trees.

JEAN DUFRENOY, STATION DE PATHOLOGIE VEGETALE,
PARIS.

[1] Badoux, H. Le pin Weymouth (*Pinus strobus*) en Suisse. *In* Jour. Forest. Suisse, Ann. 71, p. 221–227, 1920; Ann. 72, p. 86–89, p. 131–135, p. 148–152, p. 165–173, 1921.

The May number of Phytopathology was issued July 26, 1922.